Repurposing poultry effluents for irrigation in controlled environment agriculture

by

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Abstract

Rapid global population continues to drive increased demand for food production, placing significant pressure on water resources and nutrient availability. The poultry processing industry, which reflects this growth, produces approximately 901 billion liters of nutrient-rich wastewater globally each year. Although nutrients in poultry processing wastewater (PPW) holds significant potential for reuse in hydroponic systems, current industry practices treat PPW predominantly as waste. Concurrently, the agricultural sector is increasingly constrained by freshwater scarcity, rising fertilizer costs, and food safety concerns, necessitating innovative and circular resource-efficient solutions. However, there is a critical knowledge gap on the impacts of wastewater reuse such as: maintaining system performance in real-world, pilot-scale operations; crop productivity and nutrient utilization; and food safety. This dissertation introduces and validates "Poultryponics", a novel bioponics system that integrates the biological treatment of PPW with hydroponic lettuce production, by assessing pilot-scale system performance, nutrient dynamics, plant yields, and food safety.

Microalgae enhance organics removal and nitrogen transformation in wastewater treatment via photosynthetic oxygenation to heterotrophic and autotrophic bacteria. The first objective therefore, assessed the impact of microalgae on treatment performance and fate of food pathogens during long-term (>220 days) continuous pilot-scale operation (~115 L d^{-1}). The bioreactors achieved >80% soluble chemical oxygen demand (sCOD) removal but exhibited limited nitrification due to short hydraulic retention times (HRT), high organic loading, and oxygen limitation due to heterotrophic out competition. However, significant nitrification occurred in downstream hydroponic grow beds, driven by CO_2-supplemented pH modulation. UV disinfection

showed approximately 40% and 30% reductions in total coliforms and aerobic plate counts respectively, with no *Salmonella* and *Campylobacter* detection in bioreactor effluents.

The second objective of this research evaluated the effect of treated PPW on lettuce yields and plant nutrient status. Lettuce cultivated on treated PPW exhibited 58% lower shoot biomass and deficiencies in K, Mg, Ca, and Cu compared to mineral fertilizer controls. However, lettuce growth inhibition with treated PPW was mitigated through pH control (pH=7.0) and nutrient supplementation (N, P, K and micronutrients). A nitrogen mass balance revealed that although nitrogen was not limiting for plant growth, nitrogen use efficiency (nitrogen assimilated by lettuce) ranged between 3.7 and 6.3% of input nitrogen, while 65.4 – 83.0% remained in effluents. This highlights the importance of balancing nitrogen supply with targeted nutrient supplementation to enhance lettuce growth on treated PPW.

The third objective of this study addressed a critical food safety concern by evaluating the system's response to high exogenous *Salmonella* influxes (3 and 5 $\log_{10}$ CFU mL^{-1}) under simulated worst-case contamination scenarios and its implications for hydroponic lettuce. The bioreactors achieved 97.5 – 99.6% *Salmonella* removal, with no *Salmonella* detection in lettuce at the 3 $\log_{10}$ CFU mL^{-1} dosage. At the 5 $\log_{10}$ CFU mL^{-1} dosage, *Salmonella* removal in bioreactor effluent reduced to 68.4%, with *Salmonella* detection after UV treatment but not in grow beds or lettuce. Although these *Salmonella* dosages exceeded typical concentrations found in PPW, the results demonstrate the system's capacity to manage extreme *Salmonella* contamination by maintaining non-detection in hydroponic lettuce.

To expand on the treatment performance observed in Poultryponics, the fourth objective involved a comprehensive microbial community analysis to determine the impact of bioreactor conditions (illumination, CO_2 supplementation, and pH control) on dominant microbial taxa

involved in organics removal, nutrient transformation, plant growth promotion and food safety. The results demonstrated that Proteobacteria (<83.0% of prokaryotes) and Bacteroidetes (<20.6% of prokaryotes) dominated in all bioreactors, consistent with high sCOD removal efficiencies (>80%). Illumination and CO_2 sparging promoted *Desmodesmus* (<45.8% of eukaryotes) in the algal bioreactors but had no impact on nitrification. Relative abundance of total nitrifying genera remained low in all bioreactors (<0.03%) but were increased in hydroponic grow beds (<4.31%) due to pH-modulating effects of CO_2. Sulfuric acid-based pH control suppressed nitrifier and denitrifier abundance while enriching dissimilatory nitrate reduction to ammonium-associated taxa. Hydroponic grow beds enriched diverse plant growth-promoting bacteria with siderophore activity of approximately 0.6 ng L^{-1} deferoxamine mesylate equivalence in the algal system.

The absence of nitrification in bioreactors presented a bottleneck due to incompatible pH requirements between nitrifiers and lettuce. Therefore, the last research objective was to establish a continuous multistage bioreactor configuration to promote upstream nitrification and to assess the impact of bioreactor HRT on treatment efficiency, nitrogen dynamics and microbial communities. The bioreactors achieved organics removal (80 – 96%) and complete nitrification (>70% of TN) at 72-hr HRT. However, nitrogen losses reached 43% of total nitrogen (TN) at 48-hr HRT, aided by denitrifier proliferation (*Zoogloea* and *Hydrogenophaga*) and the presence of carbon sources (VFAs, and amino acids).

This dissertation advances the practical implementation of bioponic systems for sustainable food production. It is the first body of work to demonstrate continuous long-term PPW treatment for safe hydroponic irrigation. The results provide a foundation for broader wastewater reuse applications across meat processing industries and field-scale agriculture within a circular bioeconomy.

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I owe a deep debt of gratitude to my family in Ghana for their love, encouragement, and understanding throughout this journey. Even though the details of my research were unfamiliar, your support never wavered, and I could not have done this without you.

Above all, I thank God for His grace and mercy over these years. In the words of Psalms 100:4 – 5, I will "*...give thanks to Him and praise His name. For the Lord is good and His love endures forever...*"

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List of Abbreviations

AOA	Ammonia-oxidizing archaea
AOB	Ammonia-oxidizing bacteria
APC	Aerobic plate count
CDC	Centers for disease control and prevention
CEA	Controlled environment agriculture
CFU	Colony forming units
CPC	Cetylpyridinium chloride
DAF	Dissolved air floatation
dCO_2	Dissolved carbon dioxide
DHW	Dry head weight
DLI	Daily light integral
DO	Dissolved oxygen
DRW	Dry root weight
DW	Dry weight
EC	Electrical conductivity
FHW	Fresh head weight
FSIS	Food safety and inspection service
HRT	Hydraulic retention time
IAA	Indole-3-acetic acid
IC	Ion chromatography
ICP-OES	Inductively coupled plasma – optical emission spectrometry

LC	Liquid chromatography
LC-MS/MS	Liquid chromatography tandem mass spectroscopy
MLSS	Mixed liquor suspended solids
NLR	Nitrogen loading rate
NMDS	Non-metric multidimensional scaling
NOB	Nitrite-oxidizing bacteria
OLR	Organic loading rate
PAA	Peracetic acid
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
PGPB	Plant growth-promoting bacteria
PPW	Poultry processing wastewater
PSU	Percent siderophore unit
qPCR	Quantitative polymerase chain reaction
sCOD	Soluble chemical oxygen demand
SIMPER	Similarity percentage breakdown analysis
SMA	Standard methods agar
SRT	Sludge retention time
TC	Total coliforms
TDW	Total dry weight
TSS	Total suspended solids
UV	Ultraviolet
VFAs	Volatile fatty acids

Chapter 1: Introduction

1.1 Background

Intensive livestock farming is vital in ensuring global food security by supporting the growing demand for animal-based protein. This demand is driven by rapid population growth, rising incomes, and urbanization [1]. Among all livestock products, poultry meat production has exhibited the fastest growth rate in the last 50 years, with an average annual rate of 5%, compared to 3.1% for pork, 1.5% for beef, and 1.7% for small ruminants [2,3]. As the global human population is projected to reach approximately 10 billion by 2050, the demand for animal-based food is expected to rise by 76% from 2007 levels [4]. Notably, poultry meat demand is anticipated to grow by 121%, outpacing other meat categories such as beef (66%) and pork (43%) [2].

The rapid growth of poultry meat production has been accompanied by a corresponding expansion of water-intensive processing operations [5]. Globally, the meat processing sector accounts for approximately 24% of the total freshwater consumption within the food and beverage industry and up to 29% of water use in the agricultural sector [6]. Specifically, water in poultry processing is primarily used for scalding (removing feathers), washing (before and after evisceration), chilling, equipment cleaning and sanitization, as well as cooling of mechanical systems such as compressors and pumps [5,7]. These processes collectively generate an estimated 901 billion liters of poultry processing wastewater (PPW) globally each year [8]. In the United States, the world's leading producer of chicken meat (20.6% of global output; 21.3 million metric tonnes) [9], annual PPW generation is estimated at approximately 250 billion liters [5,8].

PPW contains substantial concentrations of nutrients, including approximately 90 mg N L^{-1} as total nitrogen, 20 mg P L^{-1} as total phosphorus, and 98 mg L^{-1} as potassium [10], making it a

potentially valuable resource for agricultural reuse. Despite this, the use of PPW for plant irrigation remains rare and is largely limited to non-food applications such as irrigating grass [11,12]. This hesitation is partly due to the presence of foodborne pathogens in PPW, including *Escherichia coli*, *Salmonella*, and *Campylobacter*, which pose significant food safety risks [13–18]. Consequently, the prevailing industry practice has been to discharge PPW into municipal sewer systems or directly into the environment after onsite treatment. This approach leads to nutrient loss and contributes to environmental degradation such as eutrophication of surface waters.

Meanwhile, the agricultural sector faces increasing constraints due to freshwater scarcity, rising fertilizer costs, and growing societal pressure for more sustainable farming practices [19]. As natural resources for conventional fertilizer production become scarcer, interest in resource recovery strategies such as wastewater reuse has intensified [20–22]. This is especially critical given that, without improved nutrient recovery, annual global nitrogen input in food production will rise by 45% by 2050 (255 teragrams (Tg) compared to 174 Tg in 2010) [23]. One promising solution is Bioponics; an integrated system that combines biological wastewater treatment with hydroponic crop production [20,24]. Studies have shown that diverse wastewater streams can be effectively treated and reused for cultivating edible crops such as lettuce, tomatoes, etc [25–30].

Unlike well-known wastewater reuse applications such as aquaponics, which recycles nutrients from aquaculture wastewater for hydroponic vegetable production [31–33], the reuse of PPW has not been systematically studied within the context of controlled-environment agriculture (CEA). CEA, including greenhouses and indoor vertical farms, is a rapidly growing sector in urban and peri-urban food systems [34]. These systems allow for precise environmental control, and shorter supply chains [35,36]. CEA is also well-suited to validate and optimize PPW reuse through controlled, pilot-scale demonstrations before broader application to field crops.

However, PPW must be treated prior to irrigation to mitigate plant toxicity and ensure nutrient bioavailability. The effluent typically contains reduced nitrogen (~100 mg L^{-1} as total kjeldahl nitrogen), volatile fatty acids (~1000 mg L^{-1}), proteins (~450 mg L^{-1}), and lipids (~40 mg L^{-1}), which can inhibit plant growth [10,32,37]. Conventional wastewater treatment methods like the activated sludge process requires high energy input, with mechanical aeration alone accounting for 45–75% of total plant energy costs [38], while anaerobic digestion is ineffective for nutrient transformation [39]. In contrast, integrating algae into wastewater treatment offers a sustainable, low-energy solution that simultaneously removes nutrients (C, N, and P) [40], pathogens [41,42], and organic contaminants [43–45]. Algae generate oxygen via photosynthesis, supporting heterotrophic and autotrophic microbial communities for organic matter oxidation [46], pathogen suppression [42] and nutrient transformation [40,47]. The resulting algal biomass can also serve as feedstock for biofuels [48,49], soil amendment [50], and animal feed [51].

Incorporating microalgae into PPW treatment is beneficial for hydroponic irrigation, as algae promote nitrifying bacteria that convert ammonia, typically present at toxic levels in PPW into nitrate, the preferred nitrogen form for stable plant growth [32,46,47]. Nevertheless, most existing bioponic studies have been limited to short-term batch cultures [24,25,52]. To advance the practical reuse of PPW in hydroponic systems, further research and development are needed to evaluate long-term system performance of PPW treatment by integrating algae under continuous cultures. Specifically, gaps remain in our understanding of how algal-bacterial consortia perform in long term operation, their symbiotic role in nitrogen transformation, and implications for food safety. Throughout this study, butterhead lettuce was used as the model crop due to its rapid growth, economic viability in CEA, and high susceptibility to pathogen contamination, making it a suitable model for evaluating pathogens and food safety in Poultryponics.

1.2 Research objectives

This dissertation aimed to develop and assess Poultryponics; a novel, pilot-scale bioponic system for long-term treatment and reuse of PPW for safe hydroponic irrigation. The specific objectives are as follows:

Objective 1: evaluate the impact of microalgae on organics removal, nitrogen transformation, and fate of pathogens during long-term continuous treatment of PPW in pilot-scale bioreactors. (**Chapter 2**)

Objective 2: determine the effects of treated PPW on lettuce yield and plant nutrient status, and develop a nitrogen mass balance accounting for nitrogen inputs, losses, and plant uptake. (**Chapter 3**)

Objective 3: assess system's response by quantifying the fate of *Salmonella* under high exogenous *Salmonella* influxes during simulated worst-case contamination scenarios and its implications for hydroponic lettuce. (**Chapter 4**)

Objective 4: investigate the impact of bioreactor operating conditions (illumination, CO_2 sparging, and pH control) on microbial community dynamics related to organics removal, nitrification, plant growth promotion, and food safety. (**Chapter 5**)

Objective 5: implement and evaluate a continuous multistage bioreactor configuration for upstream nitrification and examine the impact of hydraulic retention time (HRT) on organics removal, nitrogen transformation, and microbial community dynamics under long-term operation. (**Chapter 6**)

1.3 Outline of dissertation

This dissertation comprises of seven chapters and a supporting appendix. Chapter 1 provides the general introduction, and the motivation for developing the Poultryponics concept. Chapter 7 presents overall conclusions and future research directions. Chapters 2 through 6 are structured as individual research articles, developed from pilot-scale experiments conducted over three years. Each chapter can be read independently as a stand-alone piece of work, following the structure of a journal article. Nevertheless, the chapters are thematically linked to form a cohesive narrative (Figure **1.1**).

1.4 Thesis output

Chapters which have been published as papers, or are currently being prepared for submission are described below:

Chapter 2:

Arthur, W., Morgan, Z., Reina Antillon, M., Drabold, E., Wells, D. E., Bourassa, D. V., Wang, Q., & Higgins, B. T. (2024). Pilot-Scale Evaluation of Poultryponics: Insights into Nitrogen Utilization and Food Pathogen Dynamics. ACS ES&T Water, *4*(9), 3964–3975. https://doi.org/10.1021/acsestwater.4c00262

Chapter 3:

Arthur, W., Morgan, Z., Inskeep, A. E., Browne, C., Wells, D. E., Bourassa, D. V., & Higgins, B. T. (2025). Assessing nitrogen recovery in Poultryponics for hydroponic lettuce production using treated poultry processing wastewater for increased nitrogen neutrality. Bioresource Technology, 422, 132227. https://doi.org/10.1016/j.biortech.2025.132227

Chapter 4:

Arthur, W., Akplah, C. K., Drabold, E., Manjankattil, S. R., Smith, J., Wells, D. E., Bourassa, D. V., & Higgins, B. T. (2025). Dosing *Salmonella* into Poultryponics: Fate of *Salmonella* during treatment of poultry processing wastewater and irrigation of hydroponic lettuce. Journal of Environmental Management, 377, 124559. https://doi.org/10.1016/j.jenvman.2025.124559

Chapter 5:

Arthur, W., Wang, Q., Drabold, E., Shanmugam, S. R., Rezaei S., Otto M., Wells, D. E., Bourassa, D. V., & Higgins, B. T. Impact of system design and operation on microbial community structure affecting organic degradation, nitrogen transformation, plant growth promotion, and food safety in Poultryponics (**Ready for submission**)

Chapter 6:

Arthur, W., Wang, Q., Gujski E., Deng J., Drabold, E., Wells, D. E., Bourassa, D. V., & Higgins, B. T. Pilot-Scale Evaluation of Sequential Bioreactor Configuration in Poultryponics: Impact of System Design on Organics Removal, Nitrogen Transformation and Microbial Communities (**Article in preparation**)

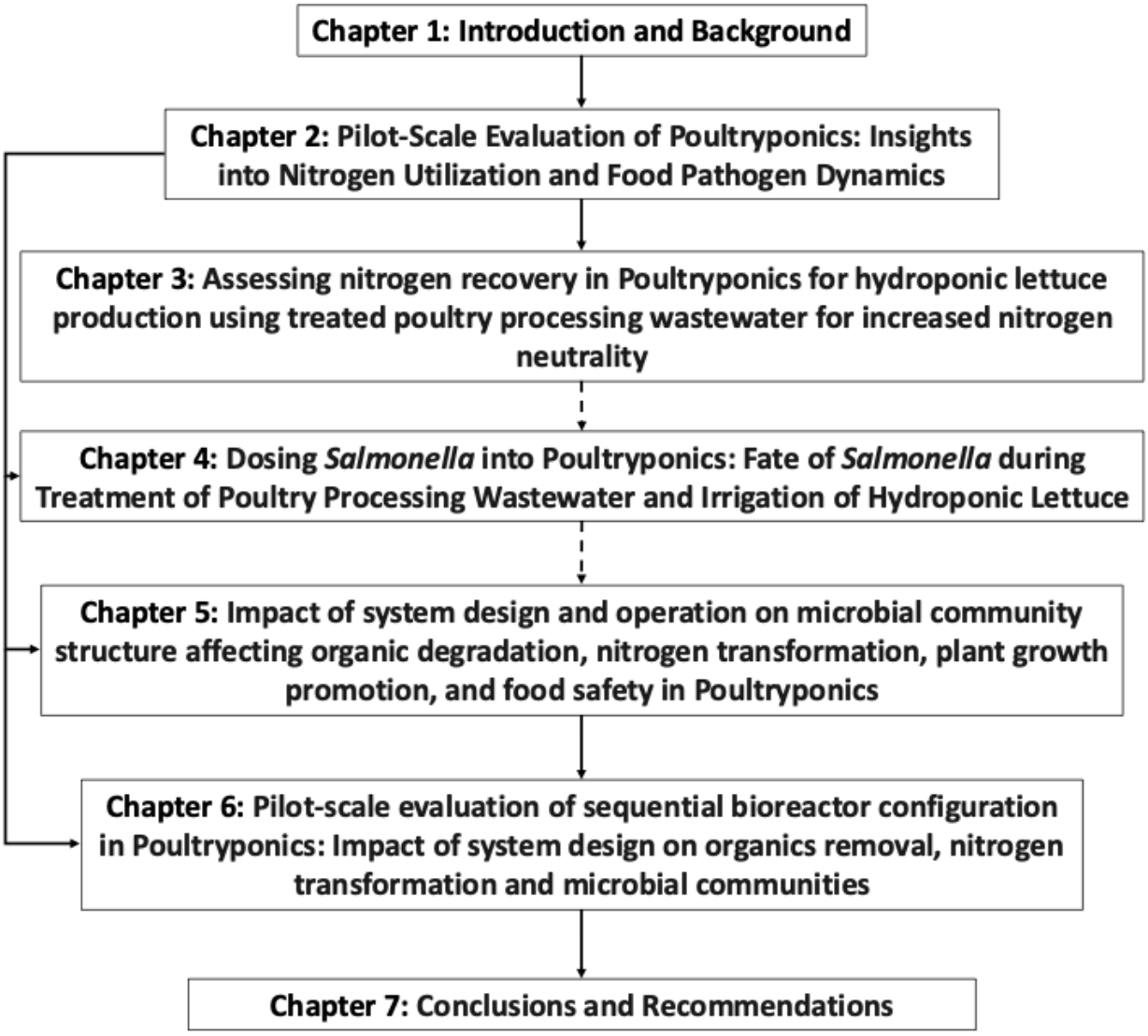

Figure 1.1: Linkages between chapters in the development and evaluation of Poultryponics for nutrient reuse, food safety, and hydroponic production using poultry processing wastewater

Chapter 2: Pilot-scale evaluation of Poultryponics: Insights into nitrogen utilization and food pathogen dynamics

Pilot-scale evaluation of Poultryponics: Insights into nitrogen utilization and food pathogen dynamics

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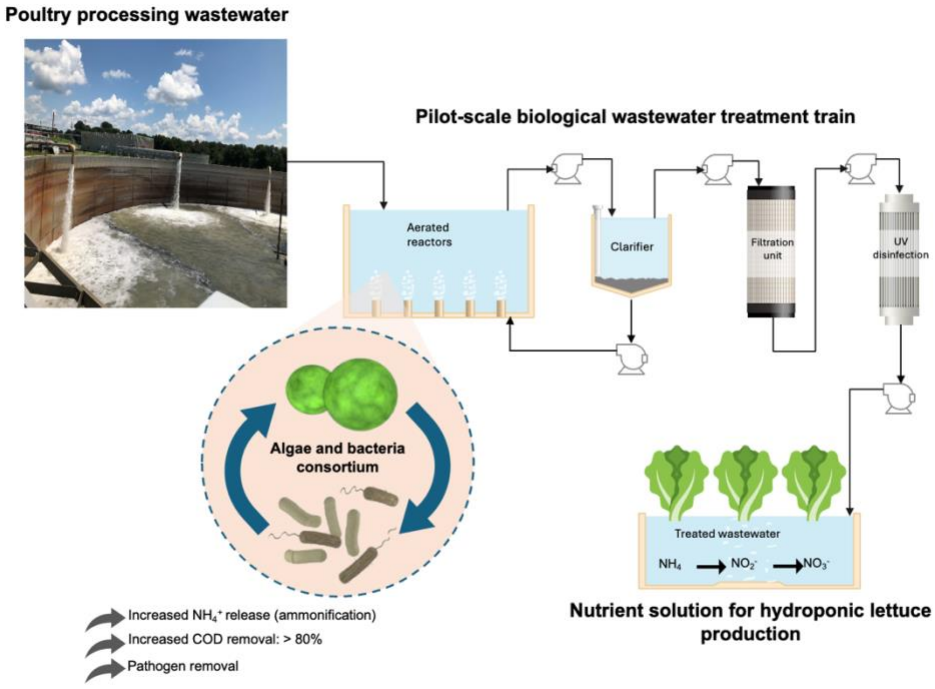
Abstract

Poultry processing wastewater (PPW) is a nutrient-rich effluent with potential for reuse in crop irrigation. This study investigated transforming PPW into a hydroponic nutrient solution using a pilot scale "Poultryponics" system operated continuously for 222 days. The system treated ~57 L d^{-1} of real PPW and consisted of bioreactors (inoculated with a consortium of microalgae and nitrifying bacteria), clarifiers, membrane filters, UV disinfection, and a deep-water hydroponic system. The system was evaluated in terms of nitrogen transformation, organic removal efficiency, and pathogen levels. Although soluble organic removal efficiencies (sCOD) were high (>80%) in all bioreactors, nitrification was limited due to high organic loading (350 - 800 mg sCOD L^{-1}), relatively short retention time (24 h), and low dissolved oxygen levels (< 3.5 mg O_2 L^{-1}). Grow beds showed significant nitrification, indicating the importance of upstream organic removal. CO_2 supplementation (0.5% v/v) in bioreactors did not promote nitrification in the bioreactors but was beneficial for nitrification in grow beds by pH-modulating effects. Microbiological analyses showed no *Salmonella* detection in or after bioreactors and substantial reductions in total coliforms (~40%) and aerobic plate counts (~30%) after UV treatment. These findings demonstrate the sustainable and safe reuse of nutrient-rich industrial effluents in agriculture.

Keywords: Bioponics; Nitrogen transformation; Pathogens; Poultry Processing Wastewater; Wastewater treatment

Graphical abstract



Synopsis

This study demonstrates the reuse of poultry processing wastewater for hydroponic irrigation, enhancing nutrient cycling and while maintaining food safety.

2.1 Introduction

The current global chicken meat production is estimated to be ~103 million tonnes, with the Unites States being the world's largest producer (21.2 million tonnes) [53]. Each processed bird generates ~26 liters of wastewater, resulting in an estimated 895 billion liters of wastewater globally in 2024 [8]. In 2021, poultry slaughtering and meat processing plants in the United States produced ~246 billion liters of wastewater at an estimated treatment cost of US $255 million [8,54]. Although poultry processing wastewater (PPW) is a rich source of nutrients such as nitrogen (74 – 89 mg N L^{-1}), phosphorus (16 – 18 mg P L^{-1}), and potassium (46 – 98 mg K L^{-1}) [10], poultry processing facilities either discharge their wastewater to city sewer systems or directly to the environment after on-site treatment [8]. Such systems aim to remove these potentially valuable nutrients rather than re-use them for beneficial applications such as plant irrigation. However, PPW contains various foodborne pathogens including *Escherichia coli* (*E. coli*), *Salmonella*, and *Campylobacter* [13,14,55] and nitrogen that is in its most reduced form (NH_4^+). Therefore, treatment is required prior to re-use to reduce the risk of foodborne disease outbreaks [14,56].

Although, advancements in wastewater treatment techniques have shown promise for mitigating nutrient pollution [57,58], water scarcity and the current supply chain challenges of inorganic fertilizer production [59] have sparked a shift toward wastewater reuse for sustainable agricultural applications[20,60,61]. A bioponic system is a technology that combines biological waste/wastewater treatment with soilless agriculture (hydroponics) [21,25,62,63]. It has been demonstrated that wastewater composed of mixtures of agricultural runoff, domestic effluent, pharmaceutical, textile, and petroleum discharges could be effectively reclaimed by biological treatment for irrigation of edible crops such as lettuce, beets, broccoli, and tomatoes [27,64]. Highly concentrated organic substrates added to the bioponic system are mineralized by microorganisms

in several compartments of the system (e.g., reactors, biofilters, hydroponic grow beds, etc.) [21,25]. In addition, organic compounds such as proteins, peptides, urea, among others are degraded while ammonium nitrogen is converted into nitrate to support crop production [25,62,65]. However, bioponic application of poultry processing wastewater (Poultryponics) has not been studied to date, perhaps due in part to concerns about pathogens. Irrigation of grass has been demonstrated with poultry processing wastewater [12] but this is considerably different from hydroponic production of food crops.

One of the most important functions in a bioponics system is nitrification because plants often cannot use organic nitrogen and are intolerant of high ammonia-N levels. Nitrification is the biological transformation of ammonium nitrogen to nitrite by ammonia oxidizing bacteria (AOB) and subsequently into nitrate by nitrite oxidizing bacteria (NOB) [39]. However, heterotrophs that mineralize ammonium nitrogen from waste effluents with high organic nitrogen loads (such as PPW) often outcompete nitrifying bacteria for oxygen at low dissolved oxygen (DO) concentrations (DO < 0.2 mg L^{-1}) [66] due to the latter's low oxygen affinities compared to heterotrophs [67].

Several studies have demonstrated that incorporating microalgae into wastewater treatment systems supplies dissolved oxygen via photosynthesis to heterotrophic and autotrophic bacteria for efficient removal of organics, and nitrification compared to conventional wastewater systems with activated sludge [40,46,47]. For example, adding *Auxenochlorella protethecoides* into PPW reportedly increased the abundance of nitrifying bacteria populations, resulting in a 4-9 fold increase in nitrate production in batch reactors [47]. Furthermore, algal photosynthetic aeration was reported to enhance the removal and inactivation of pathogenic bacteria due to high dissolved

oxygen (~15 mg L^{-1}) [68]. However, many of these studies were conducted using batch cultures over short timeframes which are not representative of commercial systems.

To fully harness the potential of reclaiming PPW as a nutrient solution for crop production, it is necessary to study such systems for extended timeframes. However, studies on the long-term effect of microalgae on nitrifying bacteria and pathogen removal in continuous systems are lacking. When bioponic and microalgae-based treatment systems are studied under continuous operation, they often utilize synthetic wastewater [64,69,70] which lacks the complex composition of real wastewater. This limits the real-world applicability of findings. Real PPW contains complex organics, food pathogens, antimicrobial agents, and persistent contaminants that impact nutrient utilization and food safety during hydroponic irrigation [64,69,70].

The overarching goal of this study is to assess the feasibility of transforming real PPW into a nutrient solution for hydroponic lettuce (*Lactuca sativa*) production at pilot scale over a 9-month period of continuous operation. The specific objectives of the work reported here were to: (a) assess the impact of microalgae on removal of organics and the transformation of nitrogen during long-term continuous operation; (b) investigate the dominant factors contributing to nitrification in the Poultryponics system; and (c) determine the fate of food pathogens (*E. coli*, *Salmonella*, and *Campylobacter*) in the various treatment compartments. The results from this study will demonstrate the feasibility of reusing PPW as a sustainable nutrient source for food crop production, highlighting nutrient cycling from wastewater, food safety and addressing potential operational challenges. It is the first study, to our knowledge, that investigates the long-term pilot scale treatment of meat processing wastewater for hydroponic irrigation. Although lettuce was cultivated in these experiments, results on lettuce growth performance and nutrient status are not

within the scope of this article. Lettuce growth performance is the subject of a subsequent publication.

2.2 Materials and Methods

2.2.1 Wastewater source

Poultry processing wastewater (PPW) was collected from a commercial broiler processing facility in the southeastern US, operating at a weekly processing capacity of ~642,000 birds. The processing facility produces ~16.7 million L of wastewater weekly based on an estimated water usage of ~26 L per bird [8]. Approximately 950 L of wastewater was withdrawn from the liquid effluent of the onsite Dissolved Air Floatation (DAF) unit and stored temporarily in a stainless steel tote for transportation. The wastewater arrived at the laboratory on the same day of collection and was pumped into a holding tank prior to treatment. A typical experiment lasted between 50 and 65 days and hence, multiple batches of fresh wastewater were collected every 10 days resulting in considerable variability in composition over time (Table **2.1**). The nutrient composition of the wastewater was similar to what was reported by Chen and Pavlostathis [10], except for soluble chemical oxygen demand (sCOD) and pH, where their study reported levels (741 – 779 mg COD L^{-1} and 4.8 – 5.4 pH) that were lower than those in our study (Table **2.1**). These differences in COD and pH could be attributed to the fact that we sampled from different processing plants with differing capacities and operations.

2.2.2 "Poultryponics" treatment system setup

The principal design and components of the pilot scale wastewater treatment system and hydroponic setup are illustrated in Figure **2.1**. PPW was continuously pumped from the holding tank into the treatment system to assess nutrient transformation, organics, and pathogen removal. Two parallel treatment trains were set up. Each treatment train was composed of parallel bioreactors (~19 L working volume) in triplicate, a ~57 L clarifier (TAMCO industries, USA), a

5-micron bag filter (PENTAIR, ES31NA410), a UV disinfection unit (HQUA–OWS–12), and a ~114 L holding tank (TAMCO industries, USA) that was used to irrigate the deep-water plant grow beds. The bioreactors were made from a sealable ~38 L glass aquarium and were inoculated with algae and nitrifying bacteria consortia from a biofloc aquaculture facility at Auburn University.

Table 2.1: Soluble nutrient composition of PPW used in each experiment.

Parameters	Average ± SD			
	Experiment 1 (n=6) *	Experiment 2 (n=5) *	Experiment 3 (n=5) *	Experiment 4 (n=5) *
sCOD (mg L^{-1})	481 ± 109	707 ± 100	408 ± 69	667 ± 173
pH	7 ± 0.1	7± 1	7 ± 0.2	7 ± 0.2
TN (mg N L^{-1})	95 ± 15	50 ± 15	44 ± 13	66 ± 34
NH_4 (mg N L^{-1})	9 ± 4	30 ± 26	25 ± 20	48 ± 26
NO_2 (mg N L^{-1})	0.1 ± 0.1	0.1 ± 0.1	0 ± 0	0 ± 0
NO_3 (mg N L^{-1})	1 ± 0.1	0.3 ± 0.4	0.1 ± 0.1	0.2 ± 0.2
TP (mg P L^{-1})	46 ± 2	29 ± 9	30 ± 6	43 ± 6
PO_4 (mg P L^{-1})	9 ± 1	13 ± 3	11 ± 3	11 ± 2
SO_4 (mg S L^{-1})	7 ± 2	10 ± 4	10 ± 3	11 ± 2
K^+ (mg L^{-1})	22 ± 6	67± 18	58 ± 10	68 ± 14
Na^+ (mg L^{-1})	167 ± 41	186 ± 37	161 ± 21	159 ± 38
Cl^- (mg L^{-1})	97 ± 22	91 ± 19	92 ± 15	91 ± 15

* Indicate the number of fresh wastewater batches collected within the experiment. See Appendix, Table A1 for detailed composition of each batch of wastewater.

The first treatment train was designed to support algal growth and is subsequently referred to as the algal-bacteria bioreactors (Treatment 1). The three parallel bioreactors in this system were sparged with 100 mL min^{-1} of air mixed with 0.5% v/v CO_2, illuminated at ~145 μmol m^{-1} s^{-1} with

LED grow lamps, and put on a 12h:12h light-dark cycle to support photosynthesis. The second treatment train was designed to suppress any algal growth and was referred to as the bacterial bioreactors (Treatment 2). These bioreactors were sparged with air and covered with a dark cloth to minimize photosynthesis and algae growth. The systems were operated for 5 weeks before the first lettuce experiment to ensure development and acclimation of the microbial communities to PPW. Each bioreactor was equipped with a pH controller (Milwaukee, MC122, USA) connected to a peristaltic pump (Stenner pumps, USA) to dose acid (0.25 M H_2SO_4) according to the experimental design (Section 2.2.3). The bioreactors were outfitted with dissolved oxygen (DO) and dissolved carbon dioxide (dCO_2) probes (Mettler Toledo InPro System), and light/temperature data loggers (HOBO, MX2202, USA).

After 24 h hydraulic retention time (HRT) in the bioreactors, the effluent flowed into the clarifier (8 h HRT) for removal of suspended solids. This HRT was selected because of the fairly high sCOD and nutrient loading in comparison to municipal sewage (240 – 290 mg sCOD L^{-1}) where an 8 h HRT is more typical [29,71]. The solids retention time (SRT) in the bioreactors was calculated at 52 and 44 days for the algal-bacteria and bacteria-only systems, respectively, due to sludge recycling from the clarifiers (Appendix). The supernatant in the clarifier was passed through the bag filters and a UV disinfection unit (~1 h HRT for both units) and then temporarily stored in a holding tank before being pumped to the plant production system. Notably, this system does not make use of costly ultrafiltration or reverse osmosis treatment steps.

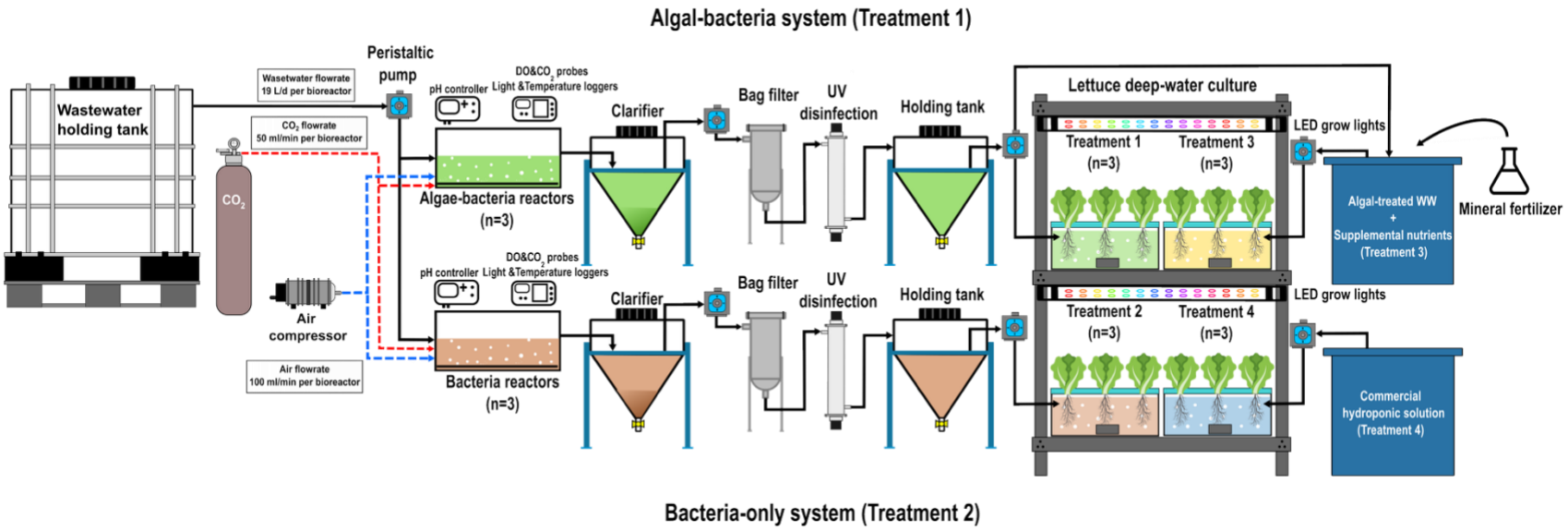


Figure 2.1: Schematic representation of pilot-scale treatment system for poultry processing wastewater and lettuce production. Treatment 1: Lettuce irrigated with effluent from the algal-bacteria treatment train, Treatment 2: Lettuce irrigated with effluent from the bacteria-only treatment train, Treatment 3: Lettuce irrigated with effluent from the algal-bacteria treatment train after supplementation with macronutrients, and Treatment 4: Lettuce irrigated with modified Sonneveld's solution.

An indoor deep-water culture hydroponic setup was constructed to evaluate plant growth parameters (Kobalt Heavy Duty Steel Shelving Unit, 5022767). Water was pumped from each treatment system to 37.9 L reservoirs (Botanicare, Vancouver, USA) via a black polyethylene tubing connected with a drip emitter and placed under a 750W LED fixture (640 μmol photons m^{-2} s^{-1}, Volt Lighting, VGL2-750-L, USA). The required water flow rate was set to 9.5 L day^{-1} to reach a 4-day HRT in the 37.9 L reservoirs [72]. Each reservoir had a drain, and a floating polystyrene board with precut holes for 6 plant grow spaces (18 plants per treatment). Aeration of the reservoirs was provided by air pumps (Hailea, ACO-9730, Guandong, China) through diffuser stones submerged at the center of the reservoir. 21-day old butterhead lettuce seedlings of green Salanova® (Johnny's seeds, Winslow, USA), were transplanted into the system.

2.2.3 Experimental design

In this study, the term "experiment" specifically referred to a complete lettuce production cycle in the Poultryponics system, spanning seedlings to transplant and harvest. However, the treatment system continued to operate even during short transition periods between lettuce experiments in order to sustain the microbial populations.

Four pilot-scale experiments were conducted from fall 2022 to summer 2023, spanning a total period of 222 days (Table **2.2**). Each experiment was designed to some extent in response to data and learning that occurred during previous experiments. To establish the baseline treatment system design and elucidate the impact of illumination of bioreactors (algal vs bacterial) on nutrient recovery and transformation, experiment 1 (days 0 – 65) was conducted. Due to robust mineralization of organic nitrogen in the bioreactors and the resulting increase in pH, varying pH control strategies were implemented in experiments 2, 3, and 4. In experiments 2 (days 65 – 110)

and 3 (days 110 – 163), 0.25M H_2SO_4 was dosed into the bioreactors to maintain a set pH of 7 while sparging 0.5% v/v CO_2 into the algal bioreactors only to promote algae growth. To assess the beneficial pH buffering effect of CO_2 sparging in bioreactors on nitrification efficiency, experiment 4 (days 163 – 222) was conducted by supplying CO_2 to both algal and bacterial bioreactors.

Table 2.2: Summary of experimental conditions used in each experiment.

Experiment	Conditions			
	Days	pH	CO_2	Lettuce
1	0 – 65	N/A	algal bioreactors only	yes
2	65 – 110	7.0	algal bioreactors only	no
3	110 – 163	7.0	algal bioreactors only	yes
4	163 – 222	6.5	all bioreactors	yes

Although data on lettuce production is not included in this article, extensive data on water quality was collected in this system as the hydroponic grow beds played an important role in further treatment of the water. As shown in Figure 2.1, lettuce was irrigated with water from the algal and bacteria treatment train (Treatment 1), bacterial train (Treatment 2), supplementation of treated water from the algal-bacteria system but supplemented with mineral fertilizer (Treatment 3), and a modified Sonneveld's solution [73] as a control (Treatment 4). For treatment 3, treated effluent from the algal-bacteria system was tested weekly for its macronutrient content (ion chromatography, see section 2.2.4) and supplemented with mineral fertilizer to match the nitrogen (266 mg L^{-1} NO_3-N), phosphorus (62 mg L^{-1} PO_4-P), and potassium (430 mg L^{-1} K^+) in the control (Treatment 4). Treated water from the algal-bacterial system was chosen for supplementation in treatment 3 because it was expected to have more robust nitrification compared to the bacteria-

only system based on previous batch culture studies [47]. It was also provided with micronutrients dosed at the same rate of those added to the control. Each treatment consisted of 3 independent deep water grow beds (experimental unit), containing 6 lettuce plants in each. The treatment scenarios elucidate the impact of algae on nutrient transformation, contaminants, and pathogen removal across the entire system.

2.2.4 Sampling and Water Quality Analyses

Water samples were collected three times per week from the various sampling points in the system, centrifuged (12,000 x g, 5 mins), and syringe filtered (0.2 μm Nylon). The supernatant was stored at -80 °C for water quality analyses. Suppressed ion chromatography on a Shimadzu Prominence Liquid Chromatography (LC) system (Shimadzu, Japan) was used to analyze soluble inorganic ions [74]. A Dionex IonPac CS16 column (4 × 250 mm, Thermo Scientific) and Dionex CERS 500e 4 mm regenerative suppressor were used to measure cations (Na^+, K^+, NH_4^+, Ca^{2+}, and Mg^{2+}). A Dionex IonPac AS22 column (4 × 250 mm, Thermo Scientific) plus regenerative suppressor (Dionex AERS 500 carbonate 4 mm) was used for the analysis of anions (Cl^-, NO_2^-, NO_3^-, PO_4^{3-}, and SO_4^{2-}). HACH assays were used for total soluble nitrogen, total soluble phosphorus, and soluble chemical oxygen demand analyses per the manufacturer's instructions. Ammonium chloride, monopotassium phosphate, and glucose were used as external validation standards for each HACH assay, respectively.

Total suspended solids (TSS) in the bioreactors were determined by centrifuging sludge samples at 4,696 x g for 5 min to form a pellet, fully decanting the free liquid, and freeze-drying the solid material. The solids content was determined by subtracting the weight of the empty tube from the tube containing the dry solid material.

2.2.5 Microbiological assessment

Fresh wastewater samples and treated effluents from the various sampling points in the system were analyzed same day to determine the microbial load. Aerobic plate count, *E. coli*, and total coliforms were enumerated as $\log_{10}$ CFU mL^{-1} by serially diluting water samples in 9 g L^{-1} sterile saline water and plating them in duplicates on their respective petrifilms (3M, USA). Water samples were screened for the presence of *Salmonella* species by adding 25 mL of water to 25 mL 2X buffered peptone water (ThermoFisher) and incubated for 24 h at 37 °C. Pre-enriched samples were inoculated into two enrichment broths: 100 µl into 9.9 mL Rappaport Vassiliadis (Hardy Diagnostics, USA) broth, and 1 mL into 9 mL Tetrathionate broth supplemented with iodine solution (Hardy Diagnostics, USA). The enrichment broths were incubated for 24 h at 42 °C, and the resultant enrichment was plated in duplicate onto Xylose Lysine Tergitol 4 agar (XLT4 agar Base, Hardy Diagnostics, USA). The XLT4 plates were incubated for 24 h at 37 °C, and colonies with black or black-centered appearance (indicating H_2S production) were considered presumptive *Salmonella* isolates. The presumptive isolates were confirmed with the 3M Molecular Detection System (MDS) which uses loop-mediated isothermal amplification for targeting *Salmonella* DNA according to the manufacturer's instruction.

For *Campylobacter* isolation, 25 mL of water was added to 25 mL 2X Bolton broth containing 5% of lysed defibrinated horse blood (Hardy Diagnostics, USA) and incubated under microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2) for 48 h at 42 °C [75]. The enrichment broth was plated directly onto Campy Cefex agar (Neogen, Michigan, USA) supplemented with cefoperazone (32 µg mL^{-1}) and incubated under microaerobic conditions for 48 h at 42 °C. Characteristic grayish and mucoid colonies were considered presumptive *Campylobacter*.

2.2.6 Statistical analyses

Data on nutrient composition are presented as treatment means ± standard deviations (SD). Averages and standard deviations of bacterial counts (transformed to $\log_{10}$ units) were conducted in Microsoft Excel. Analysis of variance and Tukey's post hoc tests were performed in R with 'car' and 'agricolae' packages to identify significant differences between treatments ($p<0.05$). Bioreactors and deep water grow beds all had 3 replicates.

2.3 Results and discussion

2.3.1 Nitrogen transformation of PPW in bioreactors

The initial ammonium nitrogen (NH_4-N) concentration in each batch of collected wastewater averaged 9 – 48 mg L^{-1}, whereas TN averaged 22 – 95 mg L^{-1} across experiments (Table **2.1**). There was a gradual increase in NH_4-N concentration in the non-aerated holding tank over time. This increase is most likely due to mineralization of organic nitrogen (ammonification) [76] as evidenced by the high influent total nitrogen (TN) compared to inorganic nitrogen concentrations (Table **2.1**). The anaerobic conditions in the holding tank likely favored heterotrophs that enzymatically degrade components like urea, free amino acids, and nucleic acids to NH_4-N [66,77].

Apart from the initial startup phase, there was only slight temporal variations in the NH_4-N concentrations in the influent wastewater and all bioreactors (Figure 2.2A). NH_4-N removal efficiency in all bioreactors ranged from -110% to 38%; where positive values suggest net ammonium removal and negative values indicate net ammonification. Net ammonium removal rates were high during the startup period of experiment 1, with 77% and 70% reductions in NH_4-N in the algal-bacteria and bacteria-only bioreactors, respectively. This could be due to direct uptake by algae and bacteria as TSS increased from 1.7 ± 0.1 g L^{-1} to 9.6 ± 1.0 g L^{-1} and 1.4 ± 0.5 g $L^{-1}$to 2.4 ± 0.3 g L^{-1} for the algal bioreactors and bacterial bioreactors respectively. Net ammonification was particularly pronounced in experiment 3, where it became apparent that some remineralization of sludge was occurring in the bioreactors as TN removal ranged from -80% to 14% (Figure **2.3**).

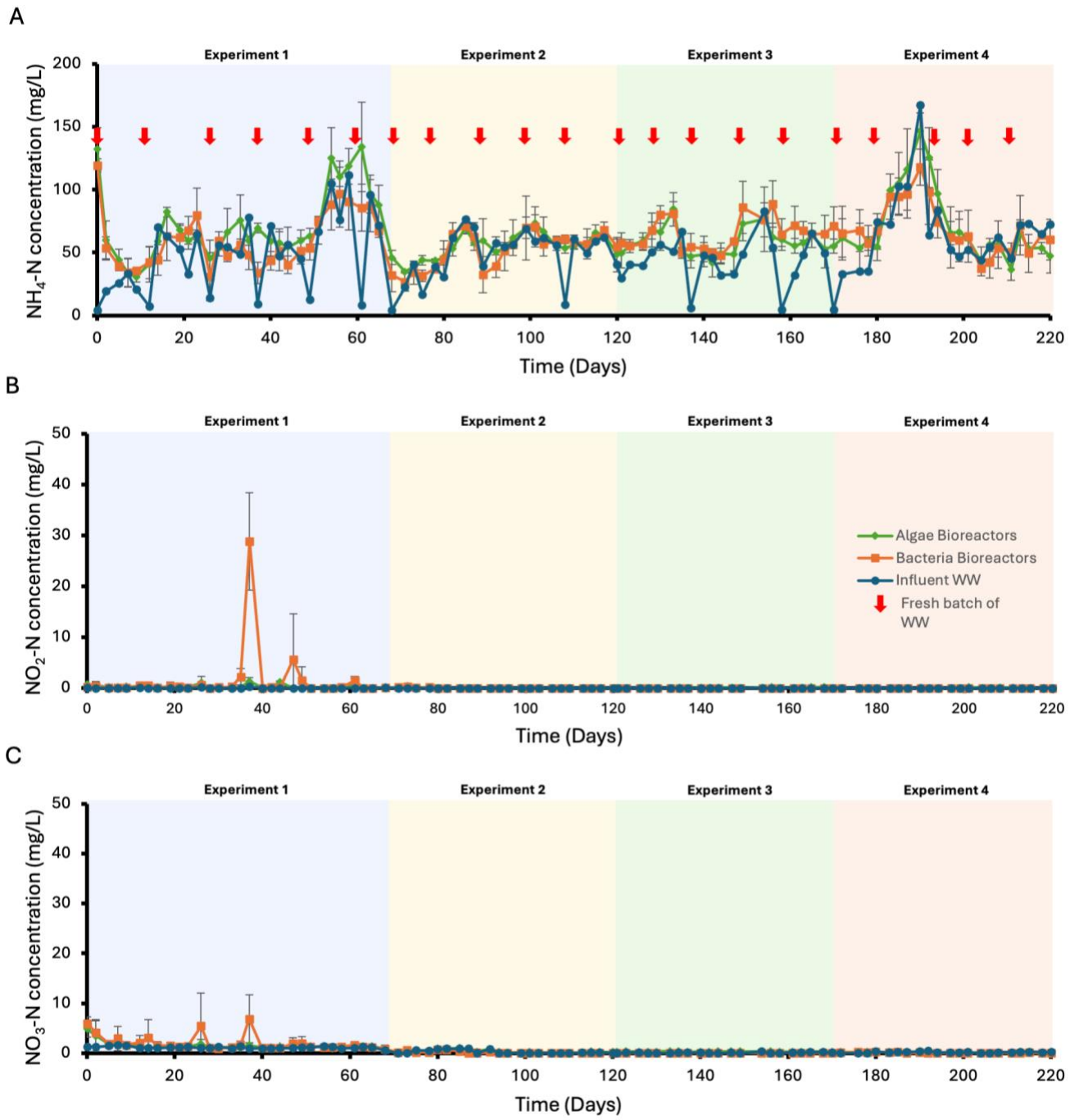


Figure 2.2: Time course plots of NH_4-N (A), NO_2-N (B), and NO_3-N (C) in wastewater and bioreactors across 4 experiments. The red arrows indicate the concentration of NH_4-N in each fresh wastewater batch (blue line). Error bars are standard deviations of 3 biological replicates.

In addition, soluble nitrogen in effluents was 10% - 20% higher than the influent nitrogen due to sludge mineralization, particularly in experiment 3 (Table **2.3**). In summary, there was little generalizable difference in the NH_4-N concentrations observed between the algal-bacteria and bacteria-only reactors over the entire study despite higher TSS in the algal system. This was likely the result of limited net biomass growth in both systems during steady operation, minimizing net assimilation of soluble N. Unlike in wastewater treatment, such an outcome is desirable in bioponics systems as nitrogen is preserved for plant production.

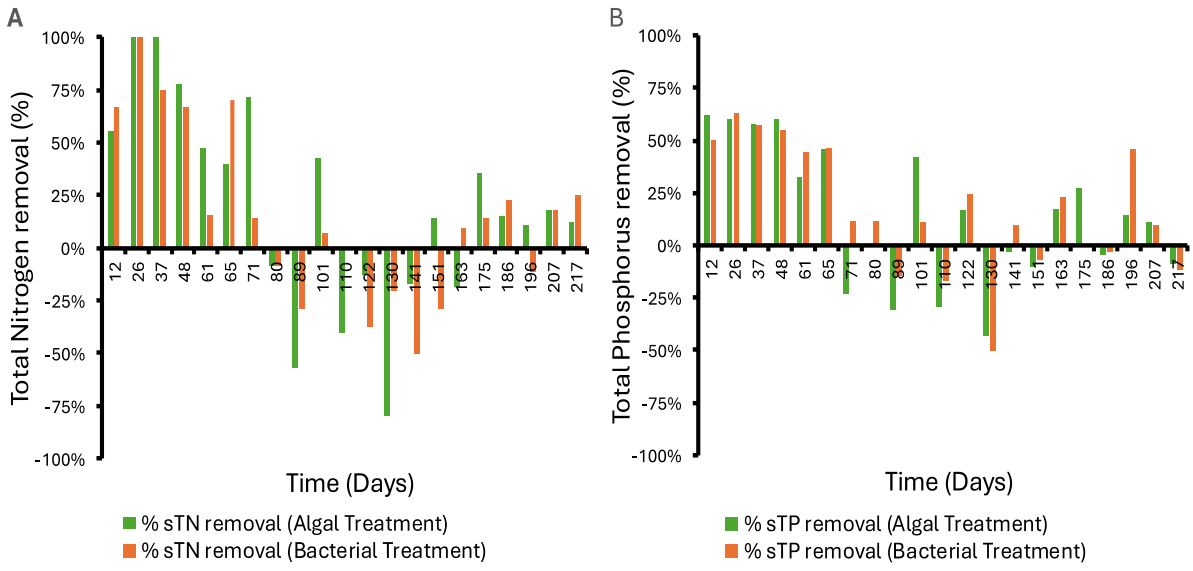


Figure 2.3: Total Nitrogen removal efficiency (%) (A) and Total Phosphorus removal efficiency (%) in treated effluent across experiments (B).

There was no substantial evidence of nitrification over the entire period of study in any of the bioreactors (Figure 2.2B&C). Although there was minimal nitrification in the bacterial bioreactors in experiment 1, it is worth mentioning that the NO_2-N and NO_3-N concentrations were only high in one of the three bioreactors. The spike in NO_2-N could be attributed to unstable microbial communities in the bioreactors during startup and low dissolved oxygen (< 3.5 mg O_2 L^{-1}; Figure **2.4**). Ammonium oxidizing archaea and bacteria (AOA and AOB) have a higher affinity for dissolved oxygen (half saturation constant, $K_{m,a}$ = 2.01 – 17.04 μM) compared to nitrite oxidizing bacteria (NOB) ($K_{m,a}$ = 5.31 – 165.63μM) [67] and could have contributed to the temporary NO_2-N spike in experiment 1.

High COD/TN ratios (11.7 – 15.2 on average) in the influent wastewater (Table **2.1**) is a contributing factor to the absence of nitrification in the bioreactors [78,79]. Continuous addition of organic matter (C/N ratio: 1.5 – 4.5) gradually increased the aerobic heterotrophic bacteria and inhibited the activity of nitrifiers (AOB and NOB) via competition for oxygen [79]. Higher DO concentration (5.1 – 7.8 mg O_2 L^{-1}; Figure **2.4**) in the algal reactors compared to the bacteria reactors did not improve nitrification because a short SRT (~5 days) in the startup phase (< day 35) could have inhibited nitrifier proliferation in the bioreactors [80]. After system startup, sludge recycling commenced, resulting in longer calculated SRTs in the range of 44 – 52 days on average. Additionally, adjustment of pH (6.5 – 7) and CO_2 sparging in bioreactors during experiments 2, 3, and 4 did not affect nitrification in the bioreactors (Figure 2.2).

Table 2.3: Summary of net removal of soluble nutrients and COD removal across experiments.

Experiment	Treatment system	N removal (%)	P removal (%)	K removal (%)	sCOD removal (%)
1	Algal	39.1	8.0	6.8	90.6
	Bacterial	48.9	7.2	1.7	91.3
2	Algal	17.6	23.6	25.2	80.2
	Bacterial	5.1	14.7	22.9	79.9
3	Algal	-9.6	4.4	-6.8	97.1
	Bacterial	-26.0	3.4	-6.3	97.5
4	Algal	34.9	10.0	20.6	82.5
	Bacterial	16.1	6.4	21.5	82.0
Average removal*	Algal	20.5%	11.5%	11.4%	87.6%
	Bacterial	11.0%	7.9%	9.9%	87.7%

*Overall average removal across all 4 experiments

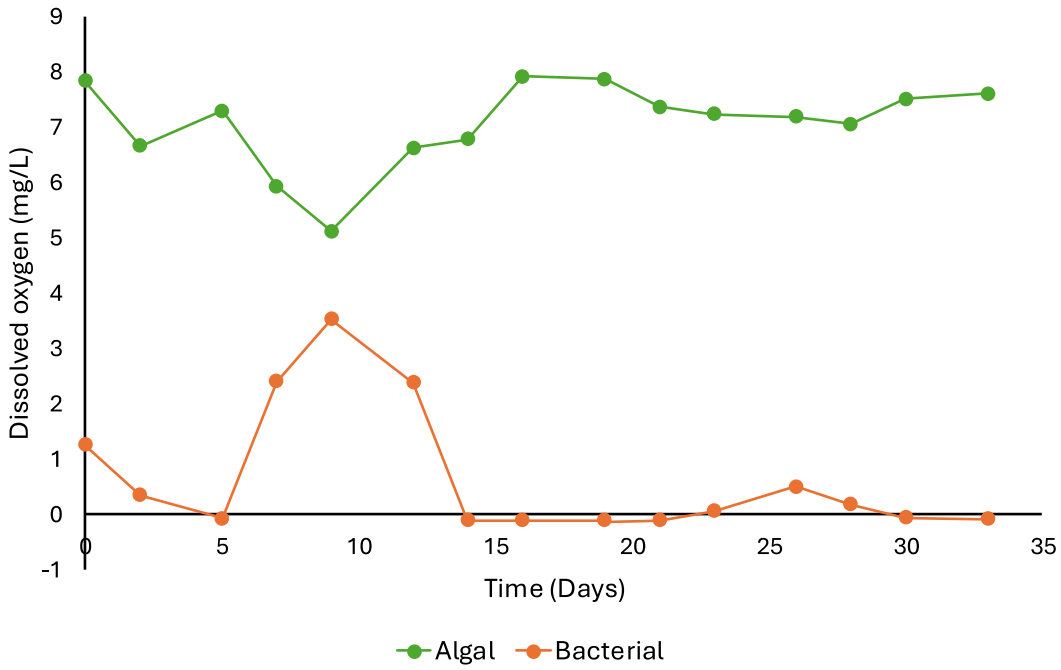


Figure 2.4: Dissolved oxygen concentration (mg/L) in bioreactors during experiment 1.

2.3.2 sCOD removal in bioreactors

The soluble chemical oxygen demand (sCOD) removal efficiency ranged between 80 and 97% across experiments (Figure 2.5). In experiment 1, ammonification and degradation of organics increased the pH to 7 – 8 in the algal bioreactors, and 8 – 9 in the bacterial bioreactors (Figure **2**.**6**). Subsequently, pH was in the range of 7.7 and 7.8 in the plant grow beds for both systems (Appendix, Table A2). These pH levels are considered high for hydroponic plant production [73]. Consequently, pH controllers that dose in sulfuric acid were implemented in the bioreactors for experiments 2 – 4. Maintaining neutral pH conditions can also help prevent NH_3 volatilization and phosphate precipitation in the bioreactors. The addition of H_2SO_4 for pH adjustment momentarily stressed the microbial community. In experiment 2, sCOD removal efficiency initially dropped to ~60% with effluent concentrations of 245 mg L^{-1} and 265 mg L^{-1} in the algal and bacterial bioreactors respectively, following the start of pH adjustment (day 71). However, sCOD removal recovered after the first week. The microbial community adapted to the pH change and sCOD removal reached ~97% throughout experiment 3 (pH controller set to 7). A similar trend of initial decline in sCOD removal followed by recovery was observed in experiment 4. The subsequent pH drop (pH controller set to 6.5) showed the same effect in the earlier days (day 186) but sCOD removal returned to ~90% for both algal and bacteria systems after acclimation.

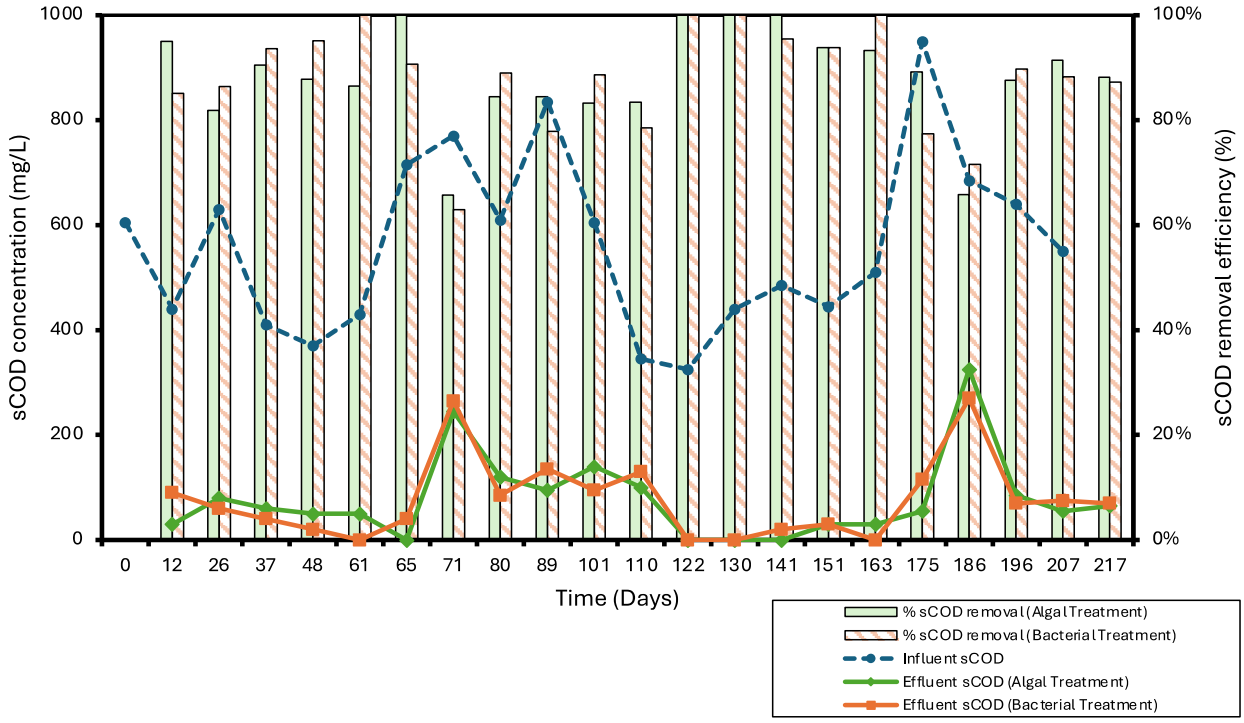


Figure 2.5: Soluble COD of influent PPW and treated effluent and COD removal efficiency (%) in treated effluent across experiments.

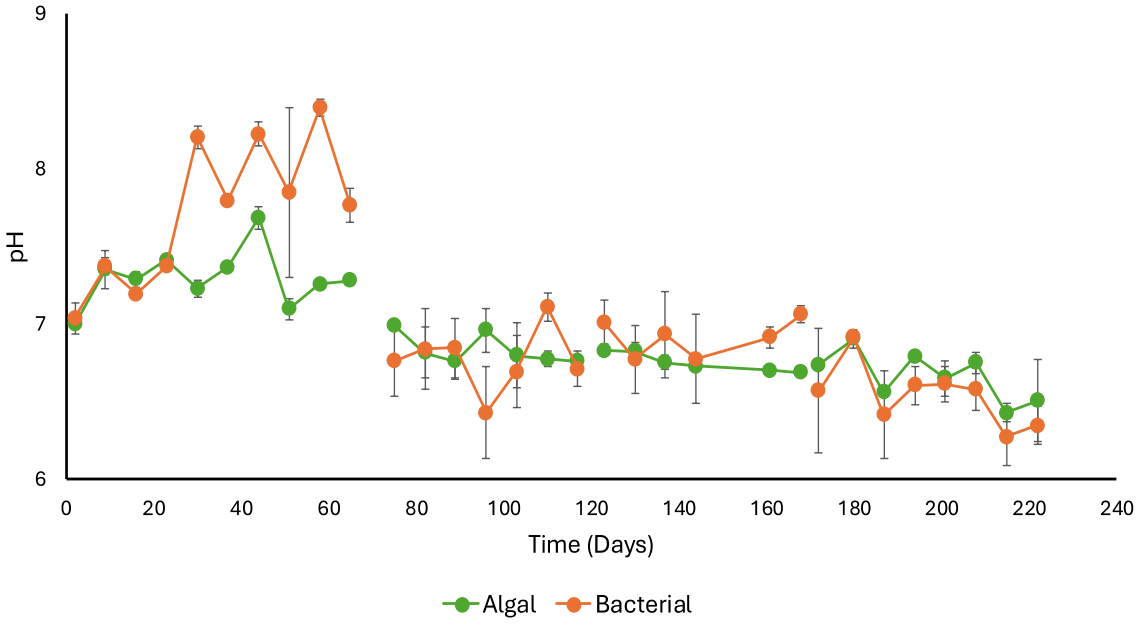


Figure 2.6: pH measurements in bioreactors across experiments.

sCOD removal in the algal bioreactors and bacteria-only bioreactors was similar throughout the study. This was true even during experiment 1 in which robust algal growth resulted in significantly higher DO concentrations (~7 mg O_2 L^{-1}, Figure **2.4**) compared to the bacterial system. In experiments 2 – 4, robust sludge recycling resulted in high biomass densities, slowing algal photosynthesis, and eliminating the DO difference between the two systems (Figure **2.7**). Despite this fact, the algal bioreactors maintained a distinct green color in contrast to the dark brown color of the bacterial bioreactors (Appendix, Figure A2).

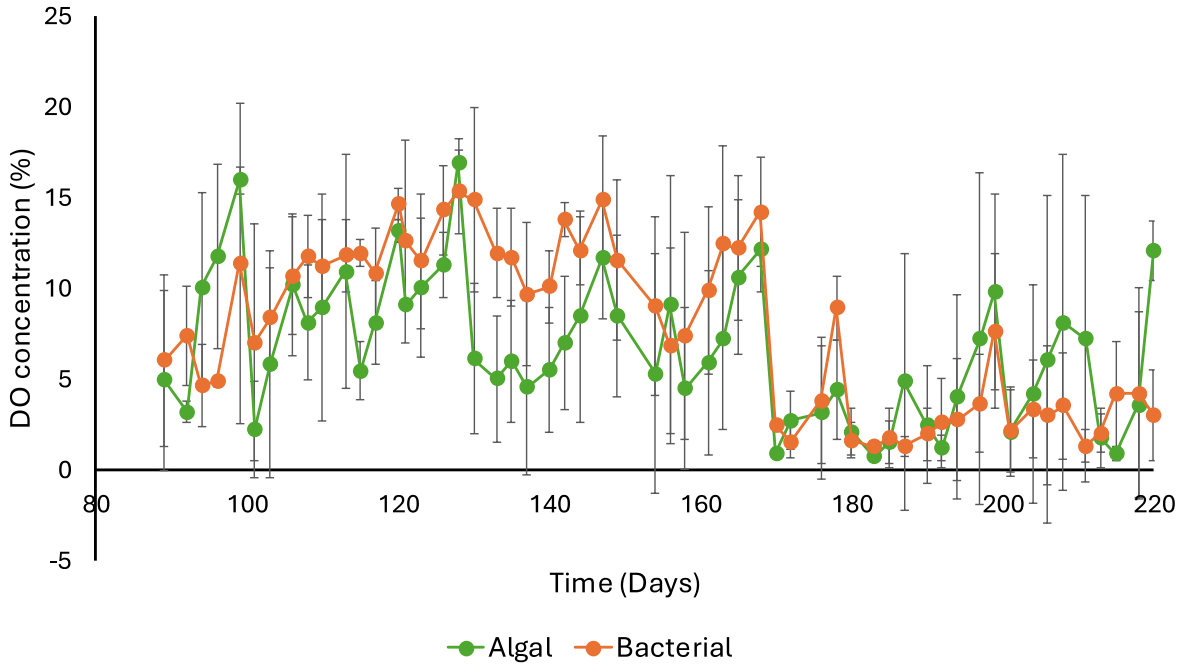


Figure 2.7: Dissolved oxygen concentration (% air saturation) in bioreactors during experiments 2, 3, 4.

Volatile fatty acids (VFAs) such as acetate and propionate can represent up to 97% of soluble COD in DAF effluents from poultry processing plants [10]. Acetic acid concentration in PPW ranged from 200 to 530 mg L^{-1} however, acetic acid in treated effluent from bioreactors reduced to 17 – 23 mg L^{-1} (~95% removal; Figure **2.8**). It is well established that both green algae and bacteria can consume VFAs, and it is likely that algae were operating in a mixotrophic and/ or heterotrophic mode [81]. Heterotrophic algal production results in net oxygen consumption, similar to bacteria systems [82]. The removal of such organic compounds from wastewater is important to prevent asphyxiation of plant roots in bioponic applications [20].

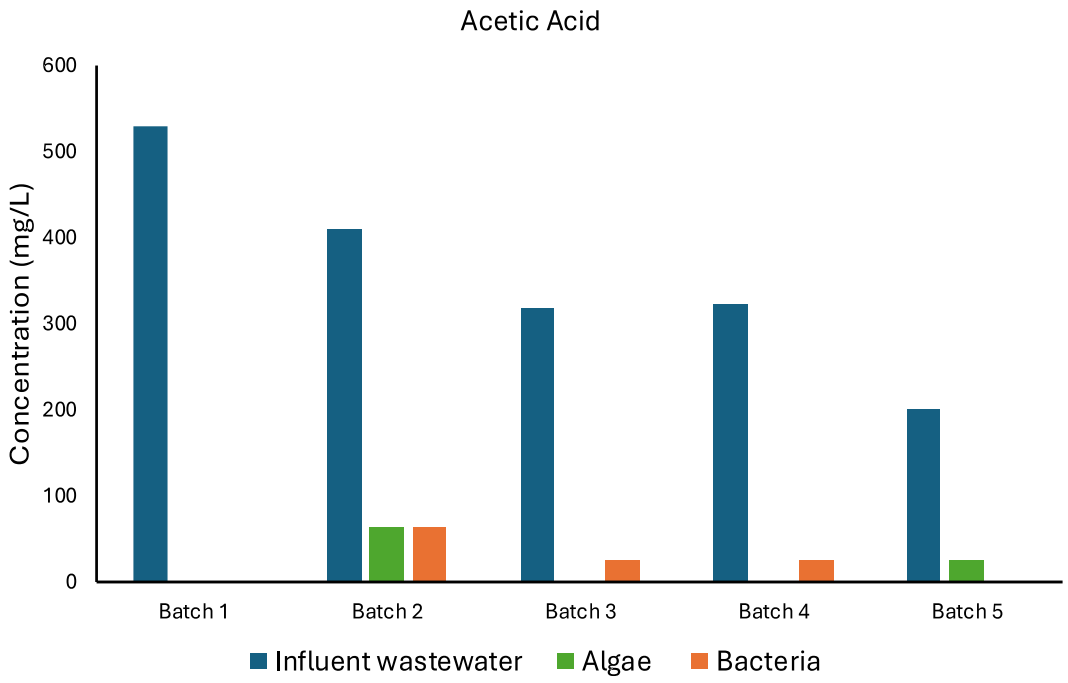


Figure 2.8: Reduction of acetic acid concentration in PPW and treated effluents from the bioreactors.

2.3.3 Nitrogen transformation of PPW in hydroponic reservoirs

Despite our initial expectation that both organics removal and nitrification would occur in the bioreactors, it soon became apparent that nitrification was taking place in the hydroponic grow beds (Figure 2.9). The combination of low sCOD and high DO levels (Figure **2.10**) due to aeration apparently favored nitrifier activity. In experiment 1, the grow beds associated with the algal and bacterial systems demonstrated comparable NH_4-N removal efficiencies (71 – 89%), primarily through cellular uptake and subsequent oxidation to NO_2-N and then NO_3-N. It is worth mentioning that the grow beds were not inoculated at the beginning of the experiments. Thus, the observed nitrification could be attributed to the bacteria that either survived the upstream treatment process or were present in the lettuce plant root zone [83].

While there was similar nitrification effect between the algal and bacterial grow beds in experiment 1, a noticeable suppression in nitrification was observed in the bacterial grow beds upon the onset of H_2SO_4 dosage in the bacterial bioreactors similar to the reduction in sCOD removal efficiency (section 3.2). Unlike the transient suppression of sCOD, however, nitrification did not recover in the bacterial system grow beds for the duration of experiments 2 and 3 (where pH was controlled in bioreactors at < 7 using H_2SO_4). During experiments 2 and 3, the algal grow beds achieved higher NH_4-N removal (76 – 81%), faster NO_2-N conversion (92 – 99%), and higher NO_3-N production (72 – 82%) compared to the bacterial system. The pH in the bacterial grow beds during these experiments was considerably lower (5.9 ± 0.6 and 5.8 ± 0.9) than in the algal system (7.0 ± 0.5 and 7.5 ± 0.2) (Appendix, Table A1). Higher dosage of H_2SO_4 was observed in the bacteria system compared to the algal system to achieve the same level of pH control in the bioreactor. This led us to believe that the CO_2 sparging in the algal reactors, initially supplied to spur algal photosynthesis, was having a meaningful secondary effect on downstream pH control.

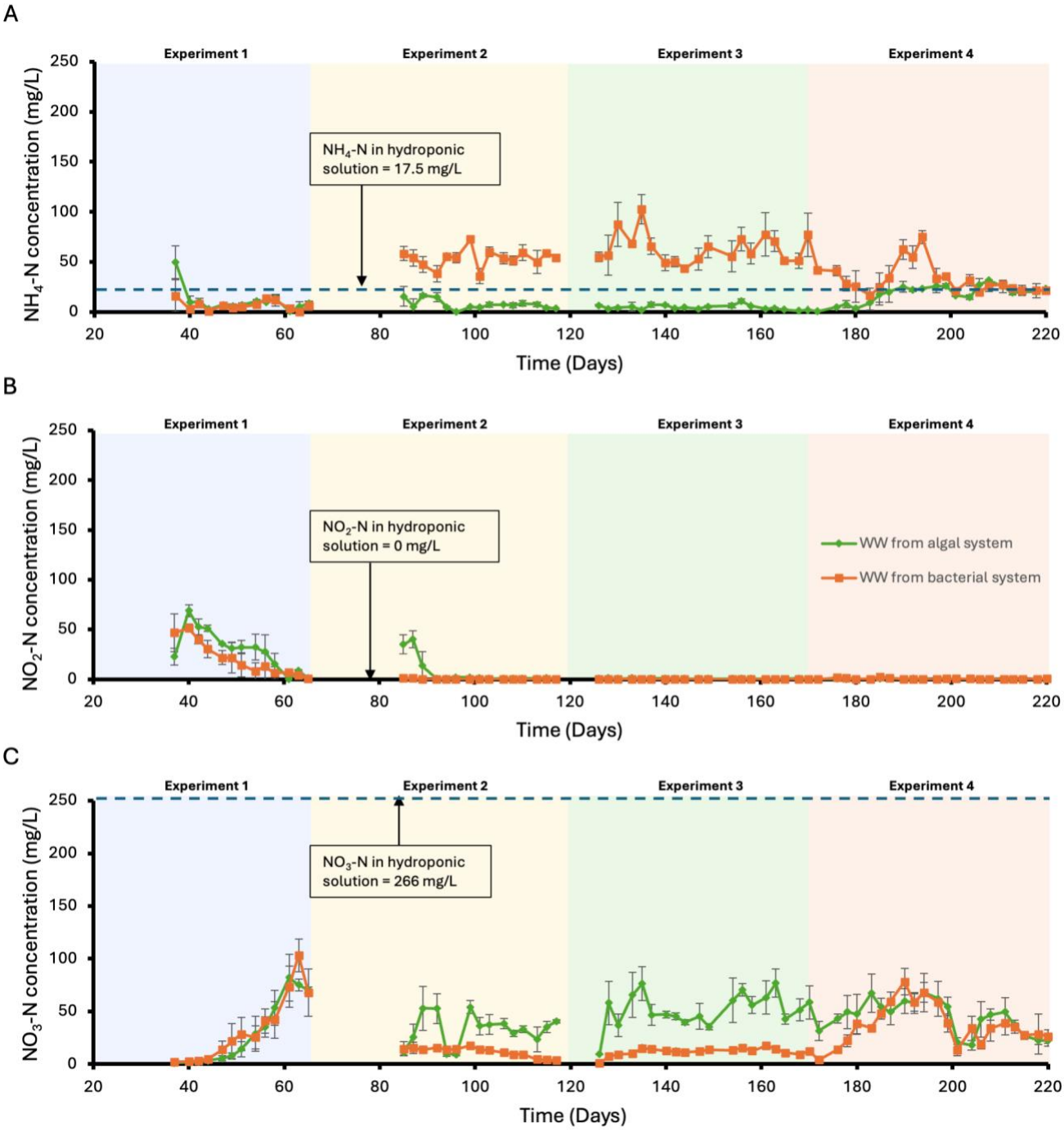


Figure 2.9: Time course plots of NH_4-N (A), NO_2-N (B), and NO_3-N (C) in grow beds across 4 experiments. The broken blue line indicates the recommended concentrations of NH_4-N, NO_2-N, and NO_3-N in lettuce hydroponic solution (Sonneveld 1994). Error bars are standard deviations of 3 biological replicates.

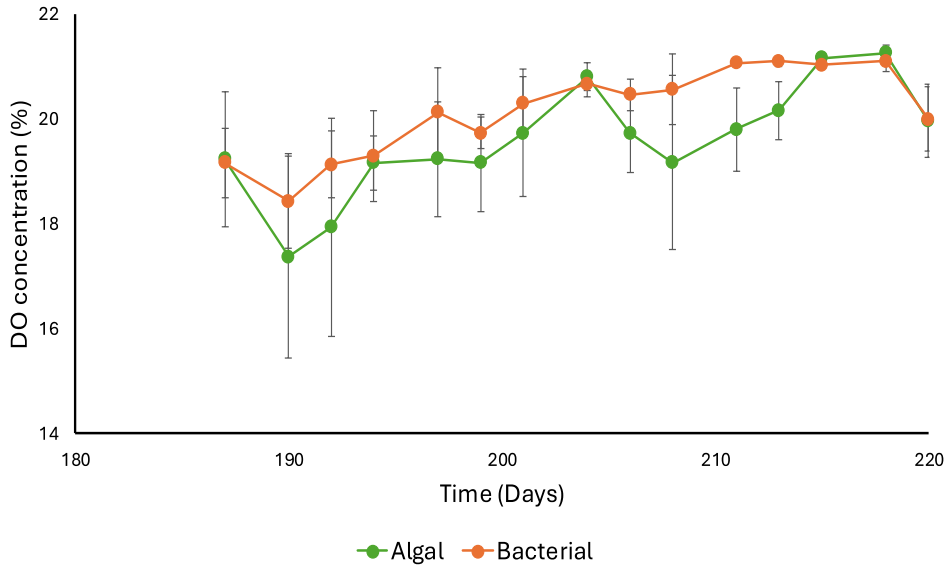


Figure 2.10: Dissolved oxygen concentration (% air saturation) in hydroponic grow beds.

CO_2 sparging at 0.5% v/v in air provided a carbonate buffer for pH regulation [71] while supporting algae growth. There was minimal H_2SO_4 dosage into the algal bioreactors in experiments 2 and 3 (SO_4-S concentration: 16 – 34 mg L^{-1} in algal system versus 138 – 232 mg L^{-1} in bacterial system; Appendix, Table A1). Therefore, while both systems had the same pH at the bioreactor stage, the algal system had achieved it in part due to dissolved CO_2. Downstream in the plant grow bed, aeration stripped much of this dissolved CO_2 from the water, resulting in higher pH in the algal system grow beds (pH = 7.0 – 7.5; Appendix, Table A1) in contrast to the bacteria system grow beds (pH = 5.8 – 5.9). As noted previously, the bacterial grow beds had achieved the "optimal" pH for plant production yet the optimal pH range for AOB is 7.9 to 8.2 and 7.2 to 7.6 for NOB [84]. Thus, nitrifier activity in the algal grow bed could be the result of a more favorable pH environment. Another possible explanation is that dissolved CO_2 supplied to the upstream algal bioreactors could have supplied dissolved inorganic carbon to autotrophic nitrifiers in the grow

beds [85,86]. In a similar vein, Soroosh et al. [71], reported that CO_2 injection favored nitrification by eliminating inhibition of NOB activities in a microalgae-bacteria treatment of municipal wastewater. While CO_2 addition can increase the growth rate of nitrifiers in a chemostat, higher concentrations (>0.4% v/v) have been found to inhibit their growth rate [87]. Nevertheless, CO_2 provision to autotrophic nitrifiers is an unlikely explanation for the nitrification disparity in our system because similar levels of nitrification were observed between the algal and bacterial grow beds in experiment 1. Another possible explanation for enhanced nitrification in algal grow beds is due to algal secretions in the upstream bioreactors. Previous batch studies on poultry processing wastewater suggest symbiosis between algae and nitrifying bacteria that is independent of dissolved oxygen production by algae [47]. However, the mechanism of symbiosis was not elucidated in that study.

To confirm if the difference in grow bed nitrification was due simply to CO_2 and pH effects or some other algal-driven mechanism, experiment 4 was conducted. This involved introducing CO_2 (0.5% v/v) into the bacterial bioreactors to match that of the algal reactors. Moreover, the bioreactor pH controllers were lowered to 6.5 in an effort to find a compromise pH that could be favorable to both plants and nitrifying bacteria in the grow bed. These changes led to a significant increase in nitrifier activity in the bacterial grow beds, similar to the algal system (Figure 2.9C), as pH in the two grow bed systems was also similar (~6.3 for algal and ~6.0 for bacterial systems; Appendix, Table A1). In our study, continuous aeration in the grow beds (DO concentration = ~21% air saturation; Figure **2.10**) facilitated the stripping of dissolved CO_2, resulting in a slight pH increase in the bacteria grow beds that could support nitrification [86]. These results underscore the importance of CO_2 in modulating pH within the grow beds, thereby influencing the overall efficiency of nitrogen transformation in hydroponic systems.

2.3.4 Nutrient composition of treated PPW and implications for hydroponic production

The analysis of nutrient composition in treated PPW showed that concentrations of NH_4-N and NO_2-N exceeded those in hydroponic solution typically used for lettuce production (Figure 2.9A&B). Specifically, NH_4-N concentration in the bacterial grow beds in experiments 2-3 ranged from 50 to 100 mg L^{-1}, significantly exceeding the 17.5 mg L^{-1} threshold that is typically used [73]. Such high ammonium levels (>25% of total N supply) can induce leaf chlorosis and oxidative stress, potentially reducing yields in hydroponic cultivation [20,73,88].

Although NO_2-N was quickly oxidized to NO_3-N in the grow beds for most of the study (NO_2-N concentrations < 2 mg L^{-1}), the concentration of NO_3-N was significantly lower than the 266 mg L^{-1} threshold typically maintained in hydroponic lettuce solutions [73]. This NO_3-N concentration suggests potential nutrient limitation for plant uptake, however, the 266 mg L^{-1} NO_3-N threshold in hydroponic solution is to ensure luxury uptake by plants without depletion. Even with NO_3-N levels below those of hydroponic solution, the plant grow beds for the algal system were never depleted of NO_3-N. Overall soluble nitrogen removal by the entire system across all experiments averaged 20.5% of influent nitrogen for the algal-bacterial treatment train and 11.0% for the bacteria treatment train (Table **2.3**).

The concentrations of Na^+ (125 – 277 mg L^{-1}) and Cl^- (75 – 132 mg L^{-1}) in treated PPW, although below the 2.34 g L^{-1} NaCl recommended limit for hydroponic nutrient solution [89], could induce salt stress in lettuce. Adhikari et al. [90] reported a 32% reduction in fresh mass of lettuce with 50 mM NaCl. Increasing salt content can create osmotic stress, interfere with the uptake of K^+ [52,89], and reduce leaf protein content [91]. Potassium levels in the treated PPW were only 58 – 85 mg L^{-1} which is an order of magnitude lower than hydroponic nutrient solution (Appendix, Table A2), and more importantly, lower than the levels of sodium. Poor K^+ uptake can lower lettuce

biomass considerably by disrupting osmotic regulation and several metabolic processes [92]. Moreover, low levels of phosphorus in treated PPW (9.4 – 16.6 mg L^{-1} PO_4-P; Appendix, Table A1) can limit lettuce growth by affecting active transport of phytohormones involved in cell division and synthesis of secondary metabolites [93]. However, PO_4-P was not depleted by plants in any of the systems. Nevertheless, consideration of nutrient supplementation (i.e. Treatment 3: treated PPW from the algal-bacteria system supplemented with mineral fertilizer) is warranted to alleviate the anticipated nutrient deficiency in lettuce irrigated with treated PPW.

Acid dosing resulted in high SO_4-S concentrations in the grow bed, most specifically in the bacterial system in experiments 2 and 3 (140 – 230 mg L^{-1}; Appendix, Table A1). High SO_4-S concentrations coupled with low NO_3-N levels can lower N uptake and affect the optimum growth of plants [73]. It is important to note that concurrent nitrification and lettuce growth in the grow beds is not ideal given the differing pH preferences, impeding the optimum growth conditions for both nitrifiers and lettuce. Nitrifying bacteria typically thrive in a neutral pH (ranging from 7 to 8) environment [84–86], whereas lettuce plants prefer a slightly acidic pH, around 5 to 6.5 [73]. Therefore, a multi-stage aerobic reactor system or extending the HRT in a single-stage reactor might be effective approaches to stimulating upstream nitrification. This approach can ensure a more consistent and suitable nutrient profile for lettuce production. Overall, while treated PPW used in this study can support lettuce growth, careful management of its nutrient composition and the cultivation environment is crucial to prevent stress or stunted growth in plants due to nutrient imbalance and suboptimal growing conditions.

2.3.5 Microbial analysis of treated PPW for hydroponic applications

The microbial load analysis conducted on PPW used in each experiment showed a consistent presence of aerobic microorganisms in the raw wastewater collected from the processing plant (Table **2.4**) Aerobic plate counts (APC) ranged from 5.24 ± 1.04 to 5.89 ± 0.42 $\log_{10}$ CFU mL^{-1}, indicating a stable aerobic microbial community. Total coliforms ranged from 3.65 ± 0.54 to 3.98 ± 1.36 $\log_{10}$ CFU mL^{-1}, with *E. coli* peaking at 0.76 ± 0.61 $\log_{10}$ CFU mL^{-1} in experiment 4. *Salmonella* was sporadically detected with enrichments (~30% of water samples collected from the poultry processing plant) while *Campylobacter* was not detected in any wastewater batches collected (Table **2.4**). The intermittent presence of *Salmonella*, albeit in low concentrations, raises concerns regarding the potential risk of pathogen transmission, especially considering that lettuce is often consumed raw [56]. Therefore, we investigated the fate of these pathogens at each stage of the Poultryponics treatment system.

Table 2.4: Microbial load of PPW used in each experiment.

$\log_{10}$ CFU mL^{-1}	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Aerobic Plate Count	5.89 ± 0.42 [a]	5.32 ± 0.49 [a]	5.24 ± 1.04 [a]	5.65 ± 0.17 [a]
Total coliforms	3.65 ± 0.54 [a]	3.87 ± 0.39 [a]	3.98 ± 1.36 [a]	3.93 ± 0.39 [a]
Escherichia coli	0.24 ± 0.43 [a]	0.59 ± 0.30 [a]	0.38 ± 0.14 [a]	0.76 ± 0.61 [a]
*Campylobacter**	0/6	0/5	0/5	0/5
*Salmonella**	2/6	1/5	0/5	1/5

For each group of bacteria, means with the same letter are not statistically different.
* (x/y) indicate the number positive of samples (x) out of the total samples collected (y).

Despite the potential exemption of PPW from the microbial quality criteria applied to agricultural water, the presence of even minimal levels of pathogens in PPW is of significant concern. Thus, implementing effective treatment methods is essential to ensure food safety, particularly for crops like lettuce [56,94]. Throughout the treatment system, considerable reductions in microbial counts were observed at various stages, starting from the non-aerated wastewater holding tank (Table **2.5**)**.** *E. coli* counts in the wastewater holding tank were reduced to zero and remained consistently low over the experimental period. Influent wastewater samples that were positive for *Salmonella*, turned out non-detectable after 72 h in the wastewater holding tank. Although *Salmonella* is a facultative anaerobe, it could have been outcompeted by other heterotrophs that were degrading organic nitrogen into NH_4-N in the non-aerated wastewater holding tank (Figure 2.2A).

In the bioreactors and clarifiers, water samples maintained high counts of APC (~6 $\log_{10}$ CFU mL^{-1}), due to biomass growth necessary for nutrient mineralization (Figure 2.2) and removal of organic contaminants (Figure 2.5). Although there was no reduction in APC across the 5-micron bag filters, total coliform counts were reduced to approximately 4 $\log_{10}$ CFU mL^{-1}following filtration, accounting for ~1 $\log_{10}$ CFU mL^{-1} reduction (Table **2.5**). The removal of fecal coliforms such as *E. coli* in wastewater treatment systems has been attributed to physical separation and predation/competition against natural microflora in closed systems with limited resources [95]. No *E. coli*, *Salmonella*, or *Campylobacter* was detected in any of the operations downstream of the wastewater holding tank.

Table 2.5: Enumeration of bacteria in the treatment system (across experiments).

Sampling point	*Escherichia coli*	Total coliforms	Aerobic Plate Count	*Campylobacter*	*Salmonella*
Wastewater tank	0 ± 0 [a]	5.28 ± 0.51 [a]	6.38 ± 0.43 [a]	nd	nd
Algal bioreactors	0 ± 0 [a]	4.52 ± 1.04 [a]	5.89 ± 0.68 [a]	nd	nd
Bacterial bioreactors	0 ± 0 [a]	4.53 ± 0.76 [a]	5.92 ± 1.22 [a]	nd	nd
Algal clarifier	0 ± 0 [a]	4.50 ± 1.08 [a]	6.07 ± 0.70 [a]	nd	nd
Bacterial clarifier	0 ± 0 [a]	4.40 ± 1.14 [a]	6.15 ± 1.38 [a]	nd	nd
Algal filter	0 ± 0 [a]	4.25 ± 0.86 [a]	5.77 ± 0.85 [a]	nd	nd
Bacterial filter	0 ± 0 [a]	3.85 ± 1.10 [a]	5.59 ± 1.31 [a]	nd	nd
Algal UV	0 ± 0 [a]	2.38 ± 0.35 [b]	3.82 ± 0.17 [b]	nd	nd
Bacterial UV	0 ± 0 [a]	2.22 ± 1.23 [b]	3.75 ± 1.61 [b]	nd	nd

Values are the means ± standard deviations of pathogen analysis conducted at the end of each experiment (n = 4). Within each group of bacteria, means with the same letter are not statistically different.
nd: not detected

UV treatment further reduced total coliform counts by ~2 $\log_{10}$ CFU mL^{-1} and APC counts by approximately 30% (Table **2.5**). The UV treatment's effectiveness in reducing coliform counts is crucial for safe hydroponic applications [96,97]. The consistent aerobic microbial population in the UV effluent, indicated by APC, coupled with downstream nitrification was beneficial for nutrient cycling (Figure 2.9). This implies that nitrifying bacteria were present in the bioreactors however, nitrification may have been hindered by organics in the wastewater (Figure **2.8**) and competition with heterotrophic bacteria.

Given the relatively low pathogen concentrations (for example *Salmonella*) in the treated PPW, follow-up studies should be conducted with exogenous dosing of high concentrations of pathogens to rigorously test the system's effectiveness under more challenging scenarios. This approach is critical for a comprehensive assessment of the system's ability to maintain microbial safety, especially for ready-to-eat vegetables.

2.4 Conclusions

This study demonstrates the potential of transforming poultry processing wastewater (PPW) into a viable nutrient solution for hydroponic lettuce cultivation. High organic loading (350 – 800 mg L^{-1} sCOD) in PPW, a short retention time (24 h), and low dissolved oxygen (< 3.5 mg O_2 L^{-1}) limited nitrification in all bioreactors. However, the bioreactors exhibited notably high sCOD removal efficiency (>80%) across all experiments. Nitrification was promoted in the hydroponic grow beds, indicating the importance of upstream organic removal. Supplementation of 0.5% v/v CO_2 into the bioreactors positively influenced the grow bed pH, benefiting the nitrification process. Nitrogen was not depleted in the plant grow bed effluent: there was an average of 20.5% N removal in the algal system and 11% removal in the bacterial system across all experiments. Pathogen analysis revealed a substantial reduction in microbial counts, particularly post-UV treatment, which reduced aerobic plate count by approximately 30%. No *E. coli*, *Salmonella*, or *Campylobacter* was detected in any of the operations downstream of the wastewater holding tank. These findings underscore the system's effectiveness in ensuring nutrient transformation and microbial safety without resorting to costly ultrafiltration. Future research should focus on challenging the system with high pathogen doses, detailed analysis of microbial communities, and improvements to system design that avoid forcing pH compromises between plants and nitrifying bacteria.

Chapter 3: Assessing nitrogen recovery in Poultryponics for hydroponic lettuce production using treated poultry processing wastewater for increased nitrogen neutrality

Assessing nitrogen recovery in Poultryponics for hydroponic lettuce production using treated poultry processing wastewater for increased nitrogen neutrality

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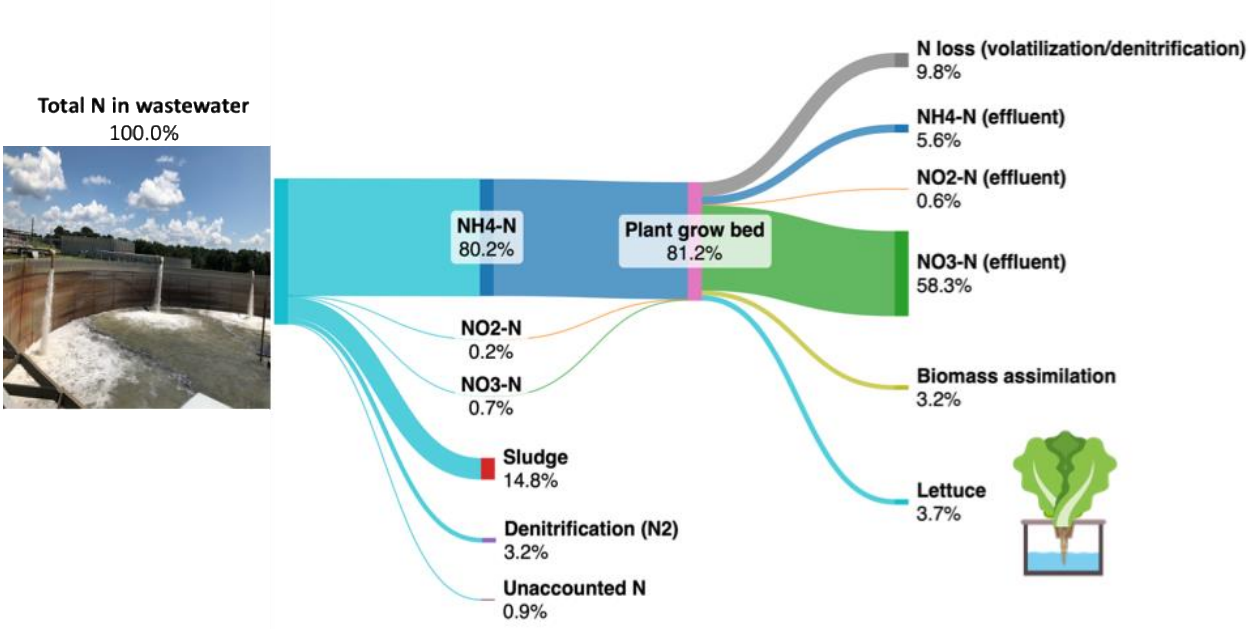
Abstract

Poultry processing wastewater (PPW) is a nutrient-rich effluent suitable for hydroponic irrigation. A nitrogen mass balance was developed for a pilot-scale Poultryponics system comprising bioreactors (algal-bacteria consortium vs. bacteria only), clarifiers, filters, UV disinfection and hydroponic grow beds to assess N recovery and lettuce production on treated PPW. Lettuce cultivated on treated PPW showed 5±1% higher root biomass, 58±10% lower shoot growth, and nutrient deficiency (K, Mg, Ca, Cu) compared to mineral fertilizer treatments ($p < 0.05$). pH control (pH=7.0) and nutrient supplementation (N, P, K and micronutrients) mitigated growth inhibition. Nitrogen was not limiting, as nitrogen utilization efficiency ranged from 3.7% – 6.3%, with 65.4% – 83.0% of input nitrogen (~213 g N) remaining in effluents, indicating the potential for a larger plant production system. These findings highlight the importance of targeted nutrient supplementation to enhance lettuce growth on treated PPW, while balancing nitrogen supply with plant demands.

Keywords: Bioponics; Lettuce; Nitrogen balance; Resource recovery; Wastewater treatment

Graphical abstract



3.1 Introduction

The rapid increase in population coupled with the rising demand for meat production is driving increased wastewater generation and placing enormous pressure on global water infrastructure [98,99]. In the poultry slaughtering, and meat processing industry alone, approximately 895 billion liters of wastewater is expected to be generated globally in 2024 [8]. In the United States, poultry processing wastewater (PPW) is either discharged to municipal wastewater treatment plants or to the environment after onsite treatment [5]. However, PPW is a nutrient-rich effluent, containing nitrogen (~90 mg N L^{-1}), phosphorus (~20 mg P L^{-1}), and potassium (~100 mg K L^{-1}) [10], presenting a significant opportunity for reuse in sustainable applications such as plant irrigation.

Bioponics is a promising technology for sustainable food production by integrating nutrient cycling (from waste/wastewater) into soilless food production (hydroponics) [20,61]. Studies have demonstrated nutrient upcycling from a variety of waste/wastewater sources (agricultural runoffs, domestic effluent, pharmaceutical, textile, and petroleum industry effluents, anaerobic digestate, food waste, chicken manure, and urine) for irrigation of edible crops such as lettuce, beets, broccoli, tomatoes, cucumber and pak choi [63,64,69].

Historically, PPW has not been reused for food crop production due to food safety concerns over pathogen contamination [14,56]. As a result, it has primarily been used for irrigating non-edible crops such as grass [12]. In a previous study, a pilot-scale biological wastewater treatment train termed "Poultryponics," was developed and operated to transform PPW into a safe nutrient solution for crop production [100]. The treatment train, which ran continuously for >220 days, demonstrated enhanced effluent quality (>80% sCOD removal), pathogen removal, and nitrogen retention after treatment (>80%) but prior to the hydroponic grow bed. Notably, pH conditions in the downstream hydroponic system strongly influenced nitrification (NH_4^+ to NO_3^-). However,

this previous study did not report on lettuce growth performance, lettuce nutrient status, or conduct a nitrogen mass balance to account for nitrogen dynamics within the system. These gaps are significant, as lettuce growth performance is expected to be significant driver of commercial viability. Additionally, minimizing nitrogen losses such as emissions of nitrous oxide (N_2O) and ammonia (NH_3) to the atmosphere, is crucial for nitrogen upcycling and maintaining the environmental benefits of the system [101].

Additionally, plants cultivated exclusively on reclaimed wastewater often exhibit limited growth due to root zone conditions such as pH, high salinity [30,69], nutrient imbalances, and the presence of growth inhibitors or heavy metals [102]. For instance, high ammonium concentrations in livestock-derived wastewater can impair the uptake of sodium, potassium, and other cations, such as zinc, magnesium, and calcium [103]. Similarly, competition between anions, such as nitrate and chloride [104], further disrupts nutrient uptake, exacerbating nutrient stress. However, studies have shown that nutrient supplementation with mineral fertilizers can alleviate growth inhibition by correcting nutrient imbalances [105].

To the best of our knowledge, no scientific literature has addressed the impact of treated PPW on hydroponic food production nor have studies accounted for a complete nitrogen budget of systems operating for extended timeframes on real PPW. Therefore, the specific objectives of this study were to: (1) evaluate the effect of the Poultryponics treatment (algal-bacterial treatment vs bacterial-only treatment) on lettuce yields and plant nutrient status; (2) elucidate the impact of root zone conditions (e.g., pH) and nutrient supplementation on lettuce yields; (3) develop a nitrogen mass balance to quantify nitrogen flows within the system, accounting for nitrogen inputs, losses, and plant uptake to support nitrogen neutrality and sustainable bioresource management.

3.2 Materials and methods

3.2.1 Description of the pilot-scale Poultryponics system

The Poultryponics system comprises a biological wastewater treatment system (~115 L d^{-1} capacity) coupled with an indoor hydroponic system for lettuce production [100]. The wastewater treatment system has two parallel treatment trains: an algal-bacterial system and a bacterial-only system. PPW collected from the effluent of a dissolved air floatation (DAF) unit at a commercial broiler processing facility was continuously pumped into the treatment train, which utilized bioreactors (illuminated in the algal system; no illumination in the bacterial system), clarifiers, bag filters, UV disinfection, water holding tanks and a deep-water system for lettuce production (Figure **3.1**). While all bioreactors were aerated (100 mL min^{-1}), the algal-bacterial bioreactors also received supplemental CO_2 (0.5% v/v in air) in order to stimulate algal growth. Details on each specific unit operation have been published previously [100].

Treated effluent from each treatment train supplied three hydroponic reservoirs (~38 L) via drip emitters at 9.5 L day^{-1} to achieve a 4-day hydraulic residence time (HRT) in the reservoirs. Each reservoir had a drain and was aerated via air stones supplied by air compressors (Hailea, ACO-9730, Guandong, China). The deepwater production system was outfitted with floating polystyrene boards with precut holes for 6 butterhead lettuce plants per reservoir (18 per treatment) and placed under a 750W LED fixture (640 mmol photons m^{-2} s^{-1}, Volt Lighting, VGL2-750-L, USA) on an 8h:16h light-dark cycle to provide a daily light integral (DLI) of 17 mol/day. All systems were set up in a climate controlled high-bay laboratory where the ambient temperature remained in the range of 20 – 25 °C.

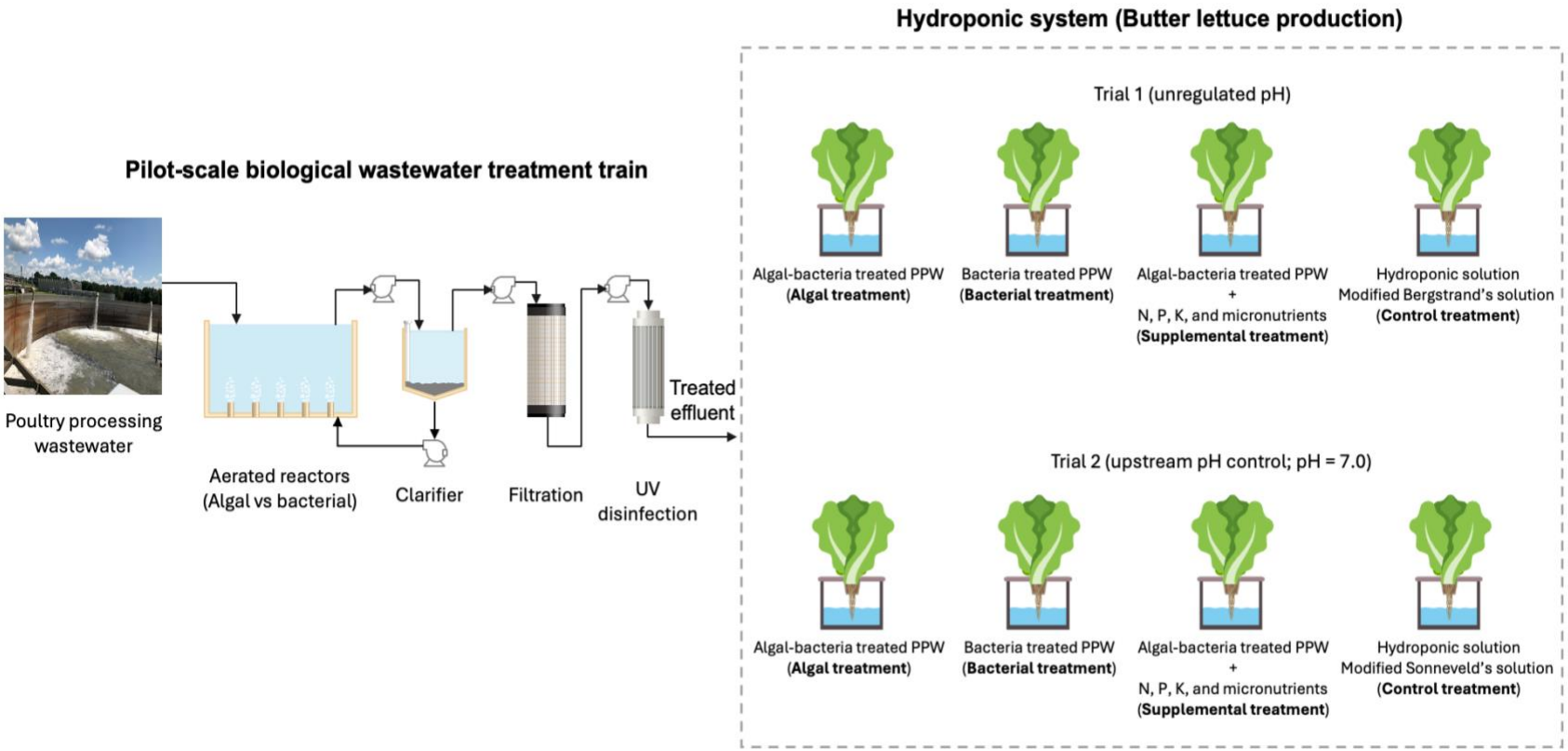


Figure 3.1: Schematic representation of Poultryponics. The two treatment trains: algal-bacteria and bacterial-only system was composed of bioreactors (3 replicates), clarifiers, bag filters, UV disinfection, water holding tank, and a hydroponic system. Lettuce was irrigated with treated wastewater from the algal-bacterial train (Treatment 1), bacterial train (Treatment 2), effluent from treatment 1 with supplemental nutrients to match N, P, K and micronutrient levels in the control solutions (Treatment 3), and commercial hydroponic solution as control (Treatment 4). Trial 1 was conducted with no pH management while Trial 2 was conducted with upstream pH control (pH controller set to 7.0). Each treatment had three hydroponic reservoirs (n=3) each with 6 butterhead lettuce (18 plants per treatment).

Lettuce seeds of green Salanova (Johnny's seeds, Winslow USA) were sown in rockwool (OASIS® Grower Solutions, Ohio USA) and grown for two weeks. The seedlings were watered with tap water for the first week and fertilized with Harrell's 20-20-20 all-purpose fertilizer (Harrell's, Florida USA) until day of transplant.

3.2.2 Experimental design

The Poultryponics system operated continuously (> 220 days) and supplied treated water for two lettuce campaigns, spanning ~35 days for each plant production campaign. Each lettuce campaign was conducted to assess the impact of system design and nutrient supplementation on lettuce yields (Figure **3**.**1**). Trial 1 was conducted to evaluate the impact of baseline system design on lettuce yields while Trial 2 evaluated the impact of upstream pH control on downstream lettuce production. In Trial 2, pH controllers were installed to dose 0.25 M H_2SO_4 into bioreactors to maintain a pH of 7. This was done to counteract the high pH observed in bioreactors in Trial 1.

Four experimental treatments were included in each lettuce trial: lettuce irrigated with treated water from the algal-bacterial treatment train (algal treatment); bacterial train (bacterial treatment); treated effluent from the algal-bacterial treatment train supplemented with mineral fertilizer (supplemental treatment), and a nutrient solution of chemical fertilizer representative of commercial hydroponic solution as control (control treatment). Each treatment was carried out in triplicate grow beds, each with 6 lettuce heads. The grow bed was treated as the experimental unit for statistical purposes (n = 3).

In Trial 1, the supplemental treatment was supplemented to match the total nitrogen, phosphorus and micronutrient concentration as those in the control while in Trial 2, the supplementation matched total nitrogen, phosphorus, potassium, and micronutrients in the control

solution. The fertilizer recipe used in the control treatment was according to a modified Bergstrand's solution [105,106] in Trial 1 and a modified Sonneveld's solution [73] in Trial 2 (Table **3.1**). The change of control solution in Trial 2 was necessary to address the lack of boron and manganese in the nutrient solution used in Trial 1. For this reason, the control lettuce between the two trials cannot be directly compared to each other. However, in both cases, they provide a point of reference to which the experimental treatments in each trial can be compared.

Table 3.1: Nutrient concentrations in the control solutions used in Trial 1 and 2.

Concentration ($mg\ L^{-1}$)	Trial 1 (modified Bergstrand's solution)	Trial 2 (modified Sonneveld's solution)
NO_3-N	150.00	266.00
NH_4-N	7.34	9.56
P	80.00	62.00
K	200.00	430.10
Mg	35.00	24.30
S	69.03	68.68
Ca	157.56	205.38
Fe	0.28	2.24
Cu	0.13	0.05
Zn	0.72	0.26
Na	13.07	17.54
Mo	0.04	0.05
B	-	0.32
Mn	-	0.27

3.2.3 Water quality analysis

Water samples were collected from all four solution holding tanks (influent to each grow bed system) and twelve hydroponic reservoirs three times per week, centrifuged, filtered and analyzed for cations (Na^+, NH_4^+, K^+, Mg^{2+}, and Ca^{2+}) and anions (Cl^-, NO_2^-, NO_3^-, PO_4^{3-}, and SO_4^{2-}) on a suppressed ion chromatography system (Shimadzu Prominence Liquid Chromatography, Shimadzu, Japan) as previously described by Q. Wang et al., (2021). The solution pH and electrical conductivity (EC) were measured three times per week with Hanna® Instruments pH and EC meter (H19813-61, Rhode Island USA). Total nitrogen (TN) was measured using HACH assays following the manufacturer's instructions. Soluble nitrogen species (NH_4-N, NO_2-N, and NO_3-N) were quantified via ion chromatography, and the difference between TN and the sum of these soluble species was attributed to organic nitrogen (org-N). Elemental analysis of water samples (Al, As, B, Cu, Fe, Mn, Mo, Pb, Si, and Zn) was performed by microwave digestion (CEM Mars 6 Microwave, Matthews, NC, USA) followed by inductively coupled plasma – optical emission spectrometry (ICP-OES) (Agilent 5800, Santa Clara, CA).

3.2.4 Lettuce growth and tissue analysis

Biometric data (fresh weight and size) from lettuce plants were recorded weekly, starting from the first week after transplant. The non-destructive weekly weight measurements were the sum of the weight of the plant (shoot and root weights), rockwool, absorbed water and weight of foam insert. The size index ([height + widest width + perpendicular weight]/3) was also recorded weekly [107]. At harvest, the weights of wet rockwool and foam insert were subtracted from the final weights recorded. Final fresh weights of lettuce heads and roots were recorded, placed in paper bags, oven-dried at 60 °C (Watlow, Missouri USA) and weighed again to determine head and root dry weights.

Biomass partitioning was used to identify morphological traits associated with plant stress [108]. Biomass partitioning was estimated as the fraction of biomass weight dedicated to head formation while root partitioning accounted for biomass allocation to root (expressed as percentage). Photosynthetic rate measurements in lettuce were conducted halfway (2 weeks after transplant) and prior to harvest using a LI-COR 6800 photosynthetic rate meter (LI-COR Biosciences, Lincoln, NE). Measurements concerning CO_2 assimilation rate, photosystem efficiency (quantum yield), transpiration rate, and stomatal conductance were recorded at the following operational chamber parameters: 550 μmol m^{-2} s^{-1} (instantaneous intensity), 30% (relative humidity) and 23°C (temperature).

Analysis of lettuce mineral nutrient content (N, P, K, Mg, Ca, S, B, Zn, Mn, Fe, and Cu) was carried out by the Ag & Environmental Services Laboratories (University of Georgia, USA). Briefly, dried lettuce samples (~0.5 g) underwent nitric acid digestion (200°C for 30 minutes) to prepare them for analysis of major plant nutrients using ICP-OES. This same lab also performed C/N analysis on dried lettuce samples using an Elementar vario MAX total combustion analyzer (Elementar, Ronkonkoma, NY, USA).

3.2.5 Nitrogen mass balance

The nitrogen mass balance was developed to quantify nitrogen dynamics across the Poultryponics system by tracking inputs, transformations, and outputs across all compartments: influent wastewater, bioreactors, clarifiers, filters, UV disinfection units, holding tanks, and hydroponic reservoirs based on data collected in Trial 2. The two parallel treatment trains (algal-bacterial and bacterial-only systems) were analyzed independently to capture the variations in nitrogen transformation specific to each configuration. The system was modeled under semi-

steady-state conditions, with the dynamics of key nitrogen forms, including ammonium (NH_4-N), nitrate (NO_3-N), nitrite (NO_2-N), and organic nitrogen (Org-N), averaged over hourly intervals to reflect system performance across compartments and until lettuce was harvested. First, the cumulative outflow of each soluble nitrogen species was determined for each individual compartment in the system through numerical integration over time (Equation 1):

$$\mathbf{N}_{c,n,outflow} = \sum_{t=0}^{t=T} \mathrm{C}_{n,c,} \boldsymbol{Q}_{c,t} \Delta \boldsymbol{t} \qquad \text{Equation 1}$$

Where N is the mass and C is the concentration of a particular soluble nitrogen species "n" (e.g. NH_4, NO_2, NO_3, org-N). The subscript "c" refers to the compartment (e.g. bioreactor, clarifier, etc), and t refers to each time step (1 h) over the course of the experiment. Q is the volumetric flow rate of water leaving the compartment at time t. Thus equation 1 was applied to every combination of soluble nitrogen species (n = 4) and compartment (c = 7). Cumulative nitrogen species flows were combined with nitrogen partitioning into sludge and lettuce to determine a total nitrogen mass outflow from each compartment (Equation 2):

$$\mathbf{N}_{c,outflow} = \sum_{n=1}^{n=4} \mathbf{N}_{n,c,outflow} + \mathbf{N}_{sludge} + \mathbf{N}_{lettuce} \qquad \text{Equation 2}$$

$N_{lettuce}$ is the nitrogen mass gained in lettuce (change in fresh weight x dry matter % x N%). N_{sludge} is the nitrogen in microbial biomass (sludge) accumulated in the bioreactors, clarifiers and filters (final weight x dry matter % x N%). As the outflow of the upstream compartment (c – 1) is the inflow of the next compartment (c), Equation 3 was used to conduct a mass balance for each compartment to estimate N losses within each.

$$\mathbf{N}_{c-1,outflow} - \mathbf{N}_{c,outflow} = \mathbf{N}_{c,loss} \quad \text{Equation 3}$$

The cumulative nitrogen flows from Equations 1 – 3 were used to develop Sankey diagrams. Nitrogen utilization efficiency (NUE) was calculated as the percentage of total nitrogen input that was assimilated into plant biomass [109–111].

The following assumptions were considered: (1) Constant water flowrate into the bioreactors (~0.8 L hr^{-1}) during the experiment. (2) Complete mixing was assumed in bioreactors due to intensive aeration hence, differences in TN in the influent and bioreactor effluent was attributed to microbial biomass assimilation and denitrification (N_2) as the dominant reductive pathway. (3) Acid dosing and high solid retention time (>40 days) resulted in sludge mineralization in bioreactors. (4) No significant biomass growth was considered in filters, UV disinfection and water holding tanks. (5) A constant elemental composition was assumed for both lettuce leaves and roots after each harvest.

3.2.6 Statistical analyses

Plant growth metrics and nutrient analysis across all treatments were compared using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to determine significant differences ($p < 0.05$). Statistical analyses were performed in R (v. 4.4.0). The grow bed was treated as the experimental unit (n = 3 per treatment).

3.3 Results and discussion

3.3.1 Lettuce biomass and morphology (Trial 1)

Significantly lower head weights were observed in lettuce samples cultivated solely on treated wastewater (algal and bacterial treatments) compared to those receiving mineral fertilizer (supplemental and control treatments) ($p < 0.05$). The fresh head weight (FHW) in the algal and bacterial treatments averaged 52 g and 58 g, respectively, while the supplemental and control treatments had FHWs of 125 g and 142 g respectively (Figure **3.2**A). These latter two treatments were not significantly different from each other in Trial 1. Other physiological metrics including total fresh weights, total dry weights, and size index were also comparable between the supplemental and control treatments (Table **3.2**). These results indicate that nutrient substitution with treated PPW (waste-derived fertilizer) can support biomass production without adverse yield impacts. Furthermore, these results suggest that treated PPW is not particularly inhibitory to lettuce growth but likely suffers from nutrient imbalances which can be corrected. This is not a trivial finding because poultry processors use a wide variety of antimicrobial chemicals for food safety including peracetic acid and quaternary ammonium compounds [112]. The impact of such compounds on lettuce growth is not well-studied but quaternary ammonium compounds have been shown to exhibit toxicity to wheat seedlings and bean plants [113,114]. While the fate of such compounds was not tracked in the present study, little adverse effect was observed from the wastewater on lettuce growth, once pH and nutrient balance were improved.

Interestingly, there was a significant increase in biomass allocation to root growth in lettuce samples cultivated solely on treated PPW compared to treatments receiving mineral fertilizer (Figure **3.2**C). Root partitioning in the algal and bacterial treatments was ~13% of dry weight, compared to ~8% of dry weight in the supplemental and control treatments ($p < 0.05$).

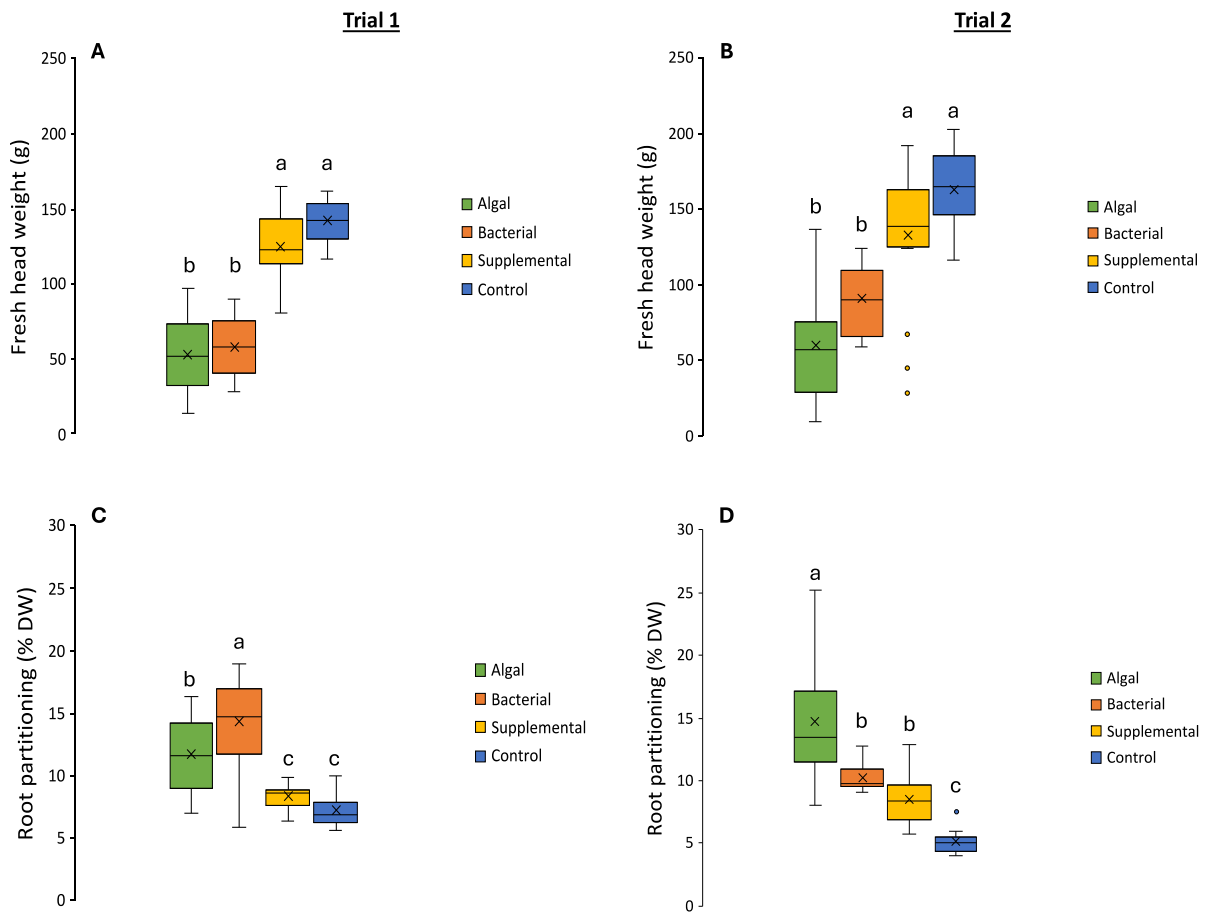


Figure 3.2: A comparison of fresh head weight (A and B) and root partitioning on % dry weight basis (C and D) of lettuce samples (n=18 per treatment) cultivated in Trial 1 with unregulated upstream pH and Trial 2 with regulated upstream pH (pH controller set to 7.0) respectively. Treatments with similar letters are not statistically different based on the Tukey's test ($p>0.05$).

Hydroponic produce cultivated on reclaimed wastewater often promotes root growth as a stress response due to unfavorable abiotic factors such as high salinity and nutrient deficiency [115,116]. While excess levels of Cu^{2+} and Zn^{2+} could potentially cause high levels of root partitioning [117], their concentrations in the treated PPW were below the critical thresholds of 0.5 mg L^{-1} for Cu^{2+} and 2 mg L^{-1} for Zn^{2+} (Table **3.3**). Additionally, such morphological effects would have been

mirrored in the supplemental treatment if present. Therefore, the high level of root partitioning is more likely attributable to the reduced uptake of key nutrients which is impacted by root-zone conditions such as pH and nutrient deficiency. For this reason, the pH and water nutrient profile were investigated in detail.

Table 3.2: Summary of physiological parameters of lettuce samples cultivated in Trials 1 and 2.

	Parameter	Treatment			
		Algal	Bacterial	Supplemental	Control
Trial 1	Total fresh weight (g)	108 ± 22 ^{b}	121 ± 17 ^{b}	185 ± 19 ^{a}	203 ± 5 ^{a}
	Fresh head weight (g)	52 ± 19 ^{b}	58 ± 12 ^{b}	125 ± 19 ^{a}	142 ± 8 ^{a}
	Fresh root weight (g)	55 ± 5 ^{a}	63 ± 5 ^{a}	60 ± 3 ^{a}	61 ± 4 ^{a}
	Dry head weight (g)	2.2 ± 0.8 ^{b}	2.8 ± 1.0 ^{b}	5.8 ± 0.6 ^{a}	6.8 ± 0.4 ^{a}
	Dry root weight (g)	0.3 ± 0.1 ^{a}	0.5 ± 0.2 ^{a}	0.5 ± 0.1 ^{a}	0.5 ± 0.1 ^{a}
	Total dry weight (g)	2.5 ± 0.9 ^{b}	3.2 ± 1.2 ^{b}	6.4 ± 0.7 ^{a}	7.3 ± 0.6 ^{a}
	Size index	5.7 ± 0.5 ^{b}	5.8 ± 0.4 ^{b}	6.7 ± 0.3 ^{a}	6.9 ± 0.2 ^{a}
Trial 2	Total fresh weight (g)	118 ± 36 ^{b}	144 ± 26 ^{b}	212 ± 12 ^{a}	214 ± 21 ^{a}
	Fresh head weight (g)	59 ± 31 ^{b}	91 ± 23 ^{b}	151 ± 11 ^{a}	167 ± 18 ^{a}
	Fresh root weight (g)	59 ± 6 ^{a}	53 ± 4 ^{ab}	61 ± 1.0 ^{a}	47 ± 3 ^{b}
	Dry head weight (g)	3.3 ± 1.3 ^{c}	4.9 ± 0.9 ^{bc}	6.5 ± 0.8 ^{b}	7.8 ± 1.1 ^{a}
	Dry root weight (g)	0.5 ± 0.1 ^{a}	0.5 ± 0.1 ^{a}	0.6 ± 0.1 ^{a}	0.4 ± 0.1 ^{a}
	Total dry weight (g)	3.8 ± 1.3 ^{b}	5.5 ± 1.0 ^{ab}	7.1 ± 0.8 ^{a}	8.2 ± 1.2 ^{a}
	Size index	5.3 ± 1.0 ^{b}	6.3 ± 0.4 ^{ab}	7.6 ± 0.3 ^{a}	7.6 ± 0.1 ^{a}

Values are means ± SD of three biological replicates (n=3) and, within a trial, means with similar letters are not statistically different based on the Tukey's test ($p>0.05$).

Table 3.3: Elemental analysis of poultry processing wastewater.

Concentration ($mg\ L^{-1}$)	Trial 1 (n = 6)	Trial 2 (n = 5)
N	95 ± 15	44 ± 13
Al	0.24 ± 0.08	<0.05
As	<0.05	<0.05
B	0.06 ± 0.01	0.04 ± 0.01
Ca	7.32 ± 0.69	6.03 ± 0.59
Cu	0.06 ± 0.01	<0.05
Fe	0.50 ± 0.04	0.17 ± 0.01
K	63.88 ± 4.90	50.30 ± 9.98
Mg	3.25 ± 0.43	2.85 ± 0.31
Mn	<0.05	0.05 ± 0.01
Mo	0.02 ± 0.00	0.02 ± 0.00
Na	145.30 ± 16.63	116.43 ± 19.63
P	12.33 ± 0.63	11.38 ± 1.15
Pb	0.06 ± 0.00	<0.05
Si	5.43 ± 0.38	5.90 ± 0.18
Zn	0.07 ± 0.01	<0.05

<0.05: below detection limit on ICP-OES.

3.3.2 Nutrient profile of treated wastewater and plant root-zone conditions (Trial 1)

The distinct morphological characteristics of plants cultivated on wastewater could be linked to nutrient uptake, which is influenced by the form and concentration of nutrients and rhizosphere conditions (pH and EC). The electrical conductivity in the wastewater-only treatments was ~36% lower ($p < 0.05$) than that in the mineral treatments (Table **3.4**), corresponding to reduced concentrations of PO_4-P, K^+, and SO_4-S in treated PPW (Table **3.5**).

Table 3.4: Average nutrient concentrations, pH and electrical conductivity of treated wastewater entering (influent) and leaving (effluent) the hydroponic reservoirs in Trial 1 with unregulated upstream pH.

Parameters	Influent			
	Algal	Bacterial	Supplemental	Control
NH_4-N (mg L^{-1})	64.2 ± 18.7 [a]	54.1 ± 21.3 [a]	57.1 ± 28.9 [a]	9.7 ± 3.1 [b]
NO_2-N (mg L^{-1})	0.7 ± 0.6 [a]	1.1 ± 0.6 [a]	0.3 ± 0.2 [a]	0.0 ± 0.0 [b]
NO_3-N (mg L^{-1})	2.4 ± 2.7 [b]	1.5 ± 0.6 [b]	158.7 ± 58.3 [a]	219.0 ± 39.6 [a]
pH	7.5 ± 0.2 [a]	7.8 ± 0.1 [a]	6.9 ± 0.2 [b]	6.1 ± 0.1 [c]
Electrical conductivity (μS/cm)	1.5 ± 0.2 [b]	1.4 ± 0.1 [b]	2.8 ± 0.5 [a]	2.1 ± 0.2 [a]
Parameters	Effluent			
	Algal	Bacterial	Supplemental	Control
NH_4-N (mg L^{-1})	11.1 ± 3.6 [a]	6.7 ± 4.8 [ab]	12.8 ± 8.0 [a]	4.8 ± 2.2 [b]
NO_2-N (mg L^{-1})	29.2 ± 20.4 [a]	20.3 ± 17.0 [a]	15.4 ± 12.1 [a]	0.0 ± 0.0 [b]
NO_3-N (mg L^{-1})	29.3 ± 21.9 [b]	33.1 ± 28.2 [b]	187.7 ± 46.0 [a]	194.9 ± 32.4 [a]
pH	7.7 ± 0.2 [a]	7.8 ± 0.2 [a]	6.9 ± 0.6 [b]	6.0 ± 0.1 [c]
Electrical conductivity (μS/cm)	1.2 ± 0.1 [b]	1.2 ± 0.1 [b]	2.1 ± 0.2 [a]	2.1 ± 0.1 [a]

Values are means ± SD and means with similar letters are not statistically different based on the Tukey's test ($p>0.05$).

Similar to other livestock-derived wastewaters, NH_4-N was the dominant nitrogen source in treated PPW. The NH_4-N concentration in treated PPW ranged between 81 and 109 mg L^{-1} in the algal, bacterial and supplemental treatments, compared to 10 mg L^{-1} in control solution which received only chemical fertilizer (Table **3.4**). This greatly exceeds the recommended NH_4-N threshold (<10% of total nitrogen; ~17.5 mg L^{-1} in control treatment) for hydroponic solutions [73]. The elevated NH_4-N concentrations in treated PPW likely contributed to the observed root

morphology, as high NH_4-N levels have been associated with rapid root growth due to efficient assimilation and limited translocation of NH_4-N [103,118]. This phenomenon may also lead to preferential NH_4-N uptake in the roots, potentially outcompeting other essential cations such as K^+ and Ca^{2+} [119].

Continuous aeration in the hydroponic reservoirs resulted in robust nitrification (conversion of NH_4^+ to NO_3^-) as described in detail in previous work [100]. However, average peak NO_3-N concentrations in the algal and bacterial treatments (50 – 61 mg L^{-1}) were significantly lower ($p < 0.05$) compared to those receiving mineral fertilizer (227 – 234 mg L^{-1}, Table **3.4**). Additionally, the apparent nitrification in the hydroponic reservoirs was facilitated by neutral pH conditions and NH_4-N inflow to the grow beds due to robust upstream ammonification and organic matter degradation [100]. The pH in the algal and bacterial hydroponic reservoirs was considerably higher (7.5 – 8) than the control treatment (~6.0). The latter pH is considered to be the optimal level for hydroponic plant production (Table **3.4**). Therefore, the combined effects of lower nutrient levels and high pH values, although optimal for nitrifying bacteria, may have negatively impacted nutrient uptake and contributed to high root partitioning in lettuce. These findings highlight the need for optimized root-zone conditions when using treated wastewater.

3.3.3 Nutrient analysis of leaf tissue (Trial 1)

In order to confirm the hypothesis that nutrient imbalances in the treated wastewater were affecting lettuce, leaf tissue analysis was carried out. Despite the lower NO_3-N concentration in the algal and bacterial treatments (Table **3.4**), nutrient analysis of leaf tissues showed no significant difference in nitrogen content between treatments, indicating that nitrogen was not limiting (Table **3.6**).

Table 3.5: Average non-N nutrient concentrations of treated wastewater entering (influent) and leaving (effluent) the hydroponic reservoirs in Trials 1 and 2.

	Parameters	Influent			
		Algal	Bacterial	Supplemental	Control
Trial 1	PO_4-P (mg L^{-1})	12.0 ± 2.1 ^{b}	12.1 ± 2.4 ^{b}	47.6 ± 17.2 ^{b}	93.0 ± 18.0 ^{a}
	SO_4-S (mg L^{-1})	12.6 ± 2.3 ^{b}	13.1 ± 2.6 ^{b}	54.2 ± 29.2 ^{a}	66.4 ± 13.0 ^{a}
	K^+ (mg L^{-1})	80.2 ± 11.6 ^{b}	84.6 ± 13.7 ^{b}	246.2 ± 82.3 ^{a}	254.7 ± 40.0 ^{a}
	Na^+ (mg L^{-1})	176.7 ± 30.4 ^{a}	185.9 ± 30.2 ^{a}	136.3 ± 70.0 ^{a}	6.6 ± 2.4 ^{b}
	Cl^- (mg L^{-1})	103.0 ± 11.5 ^{a}	104.8 ± 13.3 ^{a}	97.7 ± 20.3 ^{a}	8.7 ± 1.1 ^{b}
	Parameters	Effluent			
	PO_4-P (mg L^{-1})	11.4 ± 2.0 ^{c}	12.8 ± 2.7 ^{c}	41.0 ± 19.5 ^{b}	86.1 ± 16.7 ^{a}
	SO_4-S (mg L^{-1})	12.6 ± 2.2 ^{b}	15.0 ± 2.7 ^{b}	53.3 ± 10.6 ^{a}	60.5 ± 11.3 ^{a}
	K^+ (mg L^{-1})	78.0 ± 10.6 ^{b}	85.6 ± 23.1 ^{b}	247.0 ± 26.5 ^{a}	227.5 ± 27.2 ^{a}
	Na^+ (mg L^{-1})	183.5 ± 25.9 ^{a}	197.9 ± 31.7 ^{a}	110.2 ± 71.3 ^{a}	5.6 ± 1.5 ^{b}
	Cl^- (mg L^{-1})	106.4 ± 15.4 ^{a}	113.1 ± 19.4 ^{a}	92.5 ± 20.8 ^{a}	7.0 ± 1.4 ^{b}
	Parameters	Influent			
		Algal	Bacterial	Supplemental	Control
Trial 2	PO_4-P (mg L^{-1})	13.4 ± 2.8 ^{c}	13.5 ± 2.7 ^{c}	41.0 ± 13.1 ^{b}	80.3 ± 19.8 ^{a}
	SO_4-S (mg L^{-1})	19.1 ± 6.6 ^{c}	172.3 ± 50.4 ^{a}	50.7 ± 20.3 ^{b}	43.4 ± 9.7 ^{b}
	K^+ (mg L^{-1})	72.6 ± 20.5 ^{b}	72.3 ± 16.9 ^{b}	550.5 ± 292.7 ^{a}	685 ± 211 ^{a}
	Na^+ (mg L^{-1})	204.2 ± 53.2 ^{a}	225.0 ± 59.8 ^{a}	174.8 ± 42.2 ^{a}	20.7 ± 9.7 ^{b}
	Cl^- (mg L^{-1})	98.3 ± 23.4 ^{a}	92.5 ± 16.6 ^{a}	83.8 ± 15.9 ^{a}	21.3 ± 4.5 ^{b}
	Parameters	Effluent			
	PO_4-P (mg L^{-1})	13.0 ± 2.0 ^{b}	13.6 ± 3.0 ^{b}	63.8 ± 26.4 ^{a}	81.1 ± 17.0 ^{a}
	SO_4-S (mg L^{-1})	21.3 ± 4.9 ^{d}	184.7 ± 47.0 ^{a}	62.4 ± 18.5 ^{b}	45.4 ± 7.4 ^{c}
	K^+ (mg L^{-1})	65.7 ± 13.5 ^{b}	68.2 ± 17.4 ^{b}	710.2 ± 221.8 ^{a}	658 ± 154 ^{a}
	Na^+ (mg L^{-1})	214.8 ± 43.4 ^{a}	230.3 ± 47.2 ^{a}	209.9 ± 50.0 ^{a}	19.5 ± 7.9 ^{b}
	Cl^- (mg L^{-1})	103.8 ± 18.5 ^{a}	94.1 ± 15.3 ^{a}	95.3 ± 22.1 ^{a}	18.3 ± 4.4 ^{b}

Values are means ± SD and, within a trial, means with similar letters are not statistically different based on the Tukey's test ($p>0.05$).

Similarly, phosphorus and iron were not limiting as similar levels were detected in lettuce samples across all treatments (Table **3**.**6**). Given that nitrogen and phosphorus are integral macronutrients and account for >50% of energy input in mineral fertilizer production [120], it is significant that these macronutrients (even at moderately low concentrations in treated PPW) were not the limiting factors in the wastewater-only treatments.

However, high concentrations of NH_4^+ (81 – 109 mg L^{-1}) and Na^+ (100 – 160 mg L^{-1}, Table **3**.**3**) in treated PPW likely suppressed uptake of other cations [104,121]. Significantly lower levels of K^+, Mg^{2+}, Ca^{2+}, Zn^{2+}, and Cu^{2+} was observed in lettuce grown on non-supplemented wastewater compared to the hydroponic control (Table **3**.**6**). In addition to cation competition, low uptake of these cations can also be explained by the higher than optimal wastewater pH and low observed levels of Mg^{2+}, Ca^{2+}, and K^+ in the wastewater. Media pH in the range of 5.5-6.5 is considered optimal for nutrient availability to plants in hydroponics [122]. Deficiencies in these cations could be largely rectified through supplementation of the wastewater with these nutrients exemplified by the results of the supplemented wastewater condition.

It is worth mentioning that the Mn and B were not present in the modified Bergstrand's solution used in Trial 1 (Table **3**.**1**), resulting in their lower concentrations in lettuce tissues from the supplemental and control treatments (Table **3**.**6**). Despite this, no growth defects were observed in lettuce cultivated with mineral fertilizer (Figure **3**.**2**A&C), suggesting that these micronutrients were not critical determinants of growth performance.

Table 3.6: Nutrient composition of lettuce tissue samples cultivated in Trials 1 and 2

	Nutrient	Treatment			
		Algal	Bacterial	Supplemental	Control
Trial 1	Nitrogen (%)	7.0 ± 0.2 [a]	7.1 ± 0.1 [a]	7.2 ± 0.2 [a]	6.9 ± 0.0 [a]
	Phosphorus (%)	1.1 ± 0.1 [a]	1.2 ± 0.1 [a]	1.2 ± 0.0 [a]	1.3 ± 0.0 [a]
	Potassium (%)	5.6 ± 0.4 [c]	6.3 ± 0.2 [b]	6.7 ± 0.1 [b]	8.6 ± 0.4 [a]
	Magnesium (%)	0.29 ± 0.03 [b]	0.30 ± 0.01 [b]	0.37 ± 0.02 [a]	0.41 ± 0.01 [a]
	Calcium (%)	0.83 ± 0.04 [c]	0.83 ± 0.04 [c]	1.11 ± 0.09 [b]	1.73 ± 0.04 [a]
	Sulphur (%)	0.41 ± 0.02 [ab]	0.40 ± 0.02 [ab]	0.46 ± 0.03 [a]	0.42 ± 0.01 [b]
	Boron (mg kg^{-1})	55 ± 3 [a]	51 ± 2 [a]	52 ± 2 [a]	38 ± 0.0 [b]
	Zinc (mg kg^{-1})	170 ± 29 [b]	186 ± 14 [b]	183 ± 31 [b]	346 ± 18 [a]
	Manganese (mg kg^{-1})	116 ± 13 [b]	181 ± 44 [a]	71 ± 8 [c]	24 ± 3 [d]
	Iron (mg kg^{-1})	183 ± 21 [a]	190 ± 48 [a]	165 ± 23 [a]	129 ± 16 [a]
	Copper (mg kg^{-1})	9 ± 1 [b]	9 ± 2 [b]	24 ± 2 [a]	24 ± 1 [a]
Trial 2	Nitrogen (%)	5.6 ± 0.2 [b]	6.5 ± 0.1 [a]	6.6 ± 0.1 [a]	6.7 ± 0.0 [a]
	Phosphorus (%)	1.3 ± 0.1 [a]	1.4 ± 0.1 [a]	1.4 ± 0.1 [a]	1.5 ± 0.1 [a]
	Potassium (%)	5.8 ± 0.5 [b]	5.1 ± 0.2 [b]	9.2 ± 0.1 [a]	9.5 ± 0.3 [a]
	Sodium (%)	2.7 ± 0.4 [a]	1.8 ± 0.1 [b]	0.7 ± 0 [c]	0.1 ± 0 [d]
	Chloride (%)	0.7 ± 0.5 [a]	1.3 ± 0 [a]	1.3 ± 0 [a]	1.1 ± 0.2 [a]
	Magnesium (%)	0.31 ± 0.06 [b]	0.35 ± 0.01 [ab]	0.41 ± 0.01 [a]	0.41 ± 0.0 [a]
	Calcium (%)	1.25 ± 0.27 [b]	0.47 ± 0.04 [c]	1.41 ± 0.04 [ab]	1.65 ± 0.02 [a]
	Sulphur (%)	0.32 ± 0.02 [c]	0.71 ± 0.07 [a]	0.45 ± 0.02 [b]	0.43 ± 0.01 [b]
	Boron (mg kg^{-1})	21 ± 11 [b]	62 ± 3 [a]	31 ± 6 [b]	34 ± 3 [b]
	Zinc (mg kg^{-1})	82 ± 51 [a]	92 ± 7 [a]	97 ± 3 [a]	95 ± 2 [a]
	Manganese (mg kg^{-1})	181 ± 40 [ab]	124 ± 10 [b]	203 ± 16 [a]	138 ± 22 [b]
	Iron (mg kg^{-1})	216 ± 129 [a]	330 ± 23 [a]	279 ± 25 [a]	215 ± 10 [a]
	Copper (mg kg^{-1})	4 ± 2 [b]	5 ± 1 [b]	10 ± 1 [a]	13 ± 1 [a]

Values are means ± SD of three grow bed replicates (n=3) and means with similar letters are not statistically different based on the Tukey's test ($p>0.05$). The reported nutrients for macronutrients are based on the dry weight of the lettuce tissues.

Overall, Trial 1 confirms that treated PPW can support lettuce production, with N and P not being the limiting nutrients in treated wastewater. However, the observed inhibition of head formation; an important commercial metric, could be influenced by root zone conditions, Therefore, a follow-up study was performed (Trial 2) that improved nutrient availability and root zone conditions in the hydroponic reservoirs via pH control. Furthermore, a more balanced nutrient solution (modified Sonneveld's solution) was used to elucidate the impact of certain key micronutrients (i.e. Mn and B) on lettuce growth.

3.3.4 Effect of pH control on lettuce biomass and morphology (Trial 2)

In Trial 2, similar growth patterns were observed as in Trial 1, despite the change in pH management. As before, lower yields were obtained for lettuce cultivated on treated wastewater (algal and bacterial treatments) compared to mineral fertilizer treatments (supplemental and control) ($p < 0.05$). The average FHW in the algal and bacterial treatments were 59g and 91g respectively, while the supplemental and control treatments had FHWs of 133g and 163g respectively (Figure **3**.**2**B). Notably, using the modified Sonneveld's solution resulted in a marginally higher FHW in the supplemental and control treatments compared to Trial 1 (Figure **3**.**2**A&B) but potential batch effects rule out statistical comparisons between the trials. Once again, there were no significant differences in any physiological metrics (total fresh weights, total dry weights, and size index) between the supplemental and control treatments except for fresh root weight, which may be inconclusive considering the water adherence to roots (Table **3**.**2**).

Upstream pH control led to a more optimal pH in the bacteria grow beds (pH = ~5.8, Table **3**.**7**) whereas the upstream pH control strategy was less effective in the algal grow beds (pH = ~7.5, Table **3**.**7**) due to CO_2 buffering effects [100]. Despite the unintended outcome of those control

efforts, the difference in pH led to some instructive insight into the impact of pH on lettuce growth. pH control led to nominal improvement in physiological metrics in the bacterial treatment: total dry weight (TDW), head partitioning, and size index were closer to the control, while dry head weight (DHW) was comparable to the supplemental treatment (Table **3**.**2**). While these are secondary metrics (non-commercial), they highlight the potential of pH management in mitigating the negative effects of cultivating lettuce on treated PPW. In contrast, the algal treatment did not show significant improvement in physiological metrics relative to the control (Table **3**.**2**).

Dry root weight (DRW) was not significantly different across treatments despite the apparent differences in FHW, indicating a root partitioning response as initially observed in Trial 1 (Table **3**.**2**). Nevertheless, pH control partially mitigated negative impacts on root partitioning, particularly in the bacterial treatment, where root partitioning was ~10% of dry weight compared to ~15% of dry weight in the algal treatment ($p = 0.0002$) (Figure **3**.**2**D).

Additionally, young lettuce plants cultivated in the algal treatment exhibited lower assimilation rates (carbon fixation), photosynthetic efficiencies, transpiration rates, and stomatal conductance compared to the other treatments (Table **3**.**8**). Lower photosynthetic efficiency, transpiration rates, and stomatal conductance are a general plant stress response to limit transpiration losses associated with nutrient transport and remobilization under nutrient deficient conditions [30,115] which ultimately result in divergent structural or morphological traits [123]. Although these physiological metrics became comparable between the algal and bacterial treatments in later stages of lettuce growth, they remained significantly lower than the mineral fertilizer treatments. These observations further support earlier results (Trial 1) that lettuce cultivated solely on treated PPW has quantifiable negative impacts on plant health which may be mitigated by adding supplemental nutrients hence, nutrient availability is a dominant factor affecting plant health in Poultryponics.

Table 3.7: Average nutrient concentrations, pH and electrical conductivity of treated wastewater entering (influent) and leaving (effluent) the hydroponic reservoirs in Trial 2 with regulated upstream pH (pH controller set to 7.0).

Parameters	Influent			
	Algal	Bacterial	Supplemental	Control
NH_4-N (mg L^{-1})	61.7 ± 17.1 [a]	71.5 ± 14.4 [a]	65.0 ± 20.7 [a]	16.9 ± 4.8 [b]
NO_2-N (mg L^{-1})	0.5 ± 0.2 [a]	1.1 ± 0.7 [a]	0.0 ± 0.0 [b]	0.0 ± 0.0 [b]
NO_3-N (mg L^{-1})	0.6 ± 0.5 [b]	0.0 ± 0.0 [b]	323.9 ± 142.5 [a]	400.8 ± 104.3 [a]
pH	7.4 ± 0.1 [a]	6.8 ± 0.1 [b]	6.9 ± 0.2 [b]	6.2 ± 0.1 [c]
Electrical conductivity (μS/cm)	1.2 ± 0.0 [c]	1.4 ± 0.0 [c]	3.6 ± 0.5 [a]	2.9 ± 0.2 [b]
Parameters	Effluent			
	Algal	Bacterial	Supplemental	Control
NH_4-N (mg L^{-1})	4.9 ± 2.4 [c]	63.1 ± 15.1 [a]	11.8 ± 8.2 [b]	8.2 ± 5.2 [bc]
NO_2-N (mg L^{-1})	0.5 ± 0.3 [a]	0.0 ± 0.0 [b]	0.0 ± 0.0 [b]	0.0 ± 0.0 [b]
NO_3-N (mg L^{-1})	51.5 ± 16.6 [b]	11.8 ± 3.8 [c]	475.0 ± 146.1 [a]	405.9 ± 91.7 [a]
pH	7.5 ± 0.2 [a]	5.8 ± 0.9 [b]	6.1 ± 0.7 [b]	6.0 ± 0.2 [b]
Electrical conductivity (μS/cm)	1.0 ± 0.0 [b]	1.4 ± 0.0 [b]	3.7 ± 0.3 [a]	3.1 ± 0.1 [a]

Values are means ± SD and means with similar letters are not statistically different based on the Tukey's test ($p>0.05$).

Table 3.8: Photosynthetic characteristics of young and mature lettuce plants cultivated in Trial 2 with regulated upstream pH (pH controller set to 7.0).

Parameter	Young plants			
	Algal	Bacterial	Supplemental	Control
Assimilation rate (umol m^{-2} s^{-1})	14.7 ± 0.5 [b]	17.5 ± 0.6 [a]	16.5 ± 1.3 [ab]	17.4 ± 0.2 [a]
Photosystem 2 efficiency (%)	0.44 ± 0.02 [b]	0.53 ± 0.02 [a]	0.51 ± 0.01 [a]	0.54 ± 0.01 [a]
Transpiration rate (mol m^{-2} s^{-1})	0.005 ± 0.000 [b]	0.007 ± 0.000 [a]	0.007 ± 0.001 [a]	0.007 ± 0.000 [a]
Stomatal conductance (mol m^{-2} s^{-1})	0.31 ± 0.03 [b]	0.46 ± 0.03 [ab]	0.43 ± 0.09 [a]	0.45 ± 0.03 [a]
Parameter	Mature plants			
	Algal	Bacterial	Supplemental	Control
Assimilation rate (umol m^{-2} s^{-1})	13.6 ± 1.4 [b]	16.4 ± 1.1 [ab]	17.2 ± 0.2 [a]	16.8 ± 1.3 [a]
Photosystem 2 efficiency (%)	0.43 ± 0.02 [a]	0.49 ± 0.04 [a]	0.51 ± 0.01 [a]	0.51 ± 0.05 [a]
Transpiration rate (mol m^{-2} s^{-1})	0.003 ± 0.000 [b]	0.004 ± 0.000 [a]	0.004 ± 0.000 [a]	0.004 ± 0.000 [a]
Stomatal conductance (mol m^{-2} s^{-1})	0.30 ± 0.05 [b]	0.36 ± 0.04 [ab]	0.42 ± 0.04 [a]	0.43 ± 0.03 [a]

Values are means ± SD of three biological replicates (n=3) and means with similar letters are not statistically different based on the Tukey's test ($p>0.05$).

3.3.5 Nutrient profile of treated wastewater after upstream pH control and its effect on plant root-zone conditions (Trial 2)

Upstream pH control led to noticeable changes in the nutrient profiles of treated PPW and the resulting root-zone conditions, particularly in the bacterial treatment train. Notably, NO_3-N was the most predominant form of nitrogen in the algal reservoir (as in Trial 1), while NH_4-N remained high in the bacterial reservoir (Table **3**.**7**). The average concentrations of NH_4-N and NO_3-N in the algal reservoirs were 5 mg L^{-1} and 52 mg L^{-1} respectively, whereas the bacterial reservoirs had NH_4-N and NO_3-N concentrations of 63 mg L^{-1} and 12 mg L^{-1} respectively (Table **3**.**7**). This indicates that upstream pH regulation negatively impacted nitrification in the bacterial reservoirs, which affected the nitrogen forms available to plants. Thus, the algal grow beds exhibited a better nitrogen profile than the bacterial grow beds, but the latter had the more favorable pH for lettuce. However, limited amounts of NH_4-N are known to enhance lettuce growth compared to a nitrate-only supply [124]. Thus, it is difficult to disentangle the benefits of pH versus ammonium on lettuce growth in the bacteria system.

The concentrations of other nutrients (i.e. PO_4-P and K^+) were lower in the wastewater treatments culminating in a lower average electrical conductivity (1.0 – 1.4 μS cm^{-1}) compared to the supplemental and control treatments (3.1 – 3.7 μS cm^{-1}, Table **3**.**7**). This observation mirrors the results of Trial 1 and highlights the potential nutrient limitations in Poultryponics. Interestingly, the bacterial reservoirs exhibited significantly higher concentrations of SO_4-S (185 mg L^{-1}) compared to the other treatments (21 – 62 mg L^{-1}, Table **3**.**5**). This observation results from higher sulfuric acid dosing in the upstream bacterial bioreactors to modulate pH, in contrast to the algal reservoirs where CO_2 sparging played a role in managing pH, resulting in lower sulfuric acid dosing as previously reported [100].

Although upstream pH control was less effective in the algal treatment, it may have provided some benefits to plant growth in the bacterial treatment. Lettuce in the bacterial system had significantly better photosynthesis in early growth stages compared to those in the algal system (Table **3**.**8**). However, this faded over time, resulting in final lettuce growth metrics that were not significantly different between the two treatment systems (Table **3**.**2**). This suggests that pH control can reduce but not eliminate the stress of lettuce cultivation solely on treated PPW. These results are consistent with findings from other studies on abiotic stress [125].

3.3.6 Impact of pH control on leaf tissue nutrient composition (Trial 2)

Similar to Trial 1, lettuce cultivated solely on treated PPW were deficient in several key nutrients. Lettuce from the algal treatment had significantly lower levels of N, K, Mg, Ca, S and Cu compared to the control treatment (Table **3**.**6**). Given that NO_3-N was not limiting in the algal treatment, it is likely that the high pH resulting from CO_2 buffering effect, impacted N uptake by lettuce. In the bacterial treatment, K, Mg, Ca, and Cu levels were significantly lower in leaf tissue compared to the control. It is noteworthy that these four cations were consistently low in the lettuce cultivated on wastewater across both wastewater treatments and both trials. Thus, a targeted supplementation strategy focused on these elements is merited, as opposed to the wholistic supplementation strategy used in the present study. Comparing the non-supplemented algal and bacteria systems, only nitrogen and boron were higher in the lettuce tissue of the bacteria system despite better root zone pH. The algal treatment had higher calcium uptake, suggesting that neutral pH had only minor effects on micronutrient uptake in this case. Hence, addressing nutrient deficiencies in the wastewater is the more important issue.

Another important observation was the high salinity accumulation in lettuce cultivated on treated PPW. The average Na^+ concentration in lettuce from the algal and bacterial treatments were 1.8% – 2.7% compared to 0.1% – 0.7% in the supplemental and control treatments (Table **3.6**). This accumulation of Na^+ is attributed to the high salinity of the influent wastewater (~150 mg L^{-1} Na^+, Table **3.3**). Notably, nutrient supplementation alleviated salinity stress by promoting the uptake of other cations (such as K^+, Mg^{2+}, Ca^{2+} and Cu^{2+}) instead of Na^+ in the supplemental treatment. This result highlights the interconnected nature of cation uptake [104,121] and suggests that nutrient supplementation in Poultryponics can enhance plant resilience against salinity stress.

3.3.7 Nitrogen mass balance: Assessing nitrogen recovery in Poultryponics

Poultryponics ensured high N recovery in the upstream wastewater treatment system, with NH_4-N being the primary form of nitrogen due to minimal nitrification in the bioreactors. The overall nitrogen mass balance, which includes the nitrogen distribution across all compartments, is presented in Figure **3.3** based on data collected from Trial 2.

3.3.8 Nitrogen transformation in the upstream wastewater treatment system

Nitrogen in the wastewater entering both treatment trains (algal and bacterial systems) primarily consisted of ammonium (NH_4-N) and organic nitrogen (Org-N), accounting for 54.7% and 45.2% of the influent nitrogen load respectively, with minor fractions of nitrite (NO_2-N) and nitrate (NO_3-N) (Figure **3.3**). As nitrogen flowed through the various treatment stages: bioreactors, clarifiers, filters, UV disinfection, and into the hydroponic reservoirs, a series of transformations were observed.

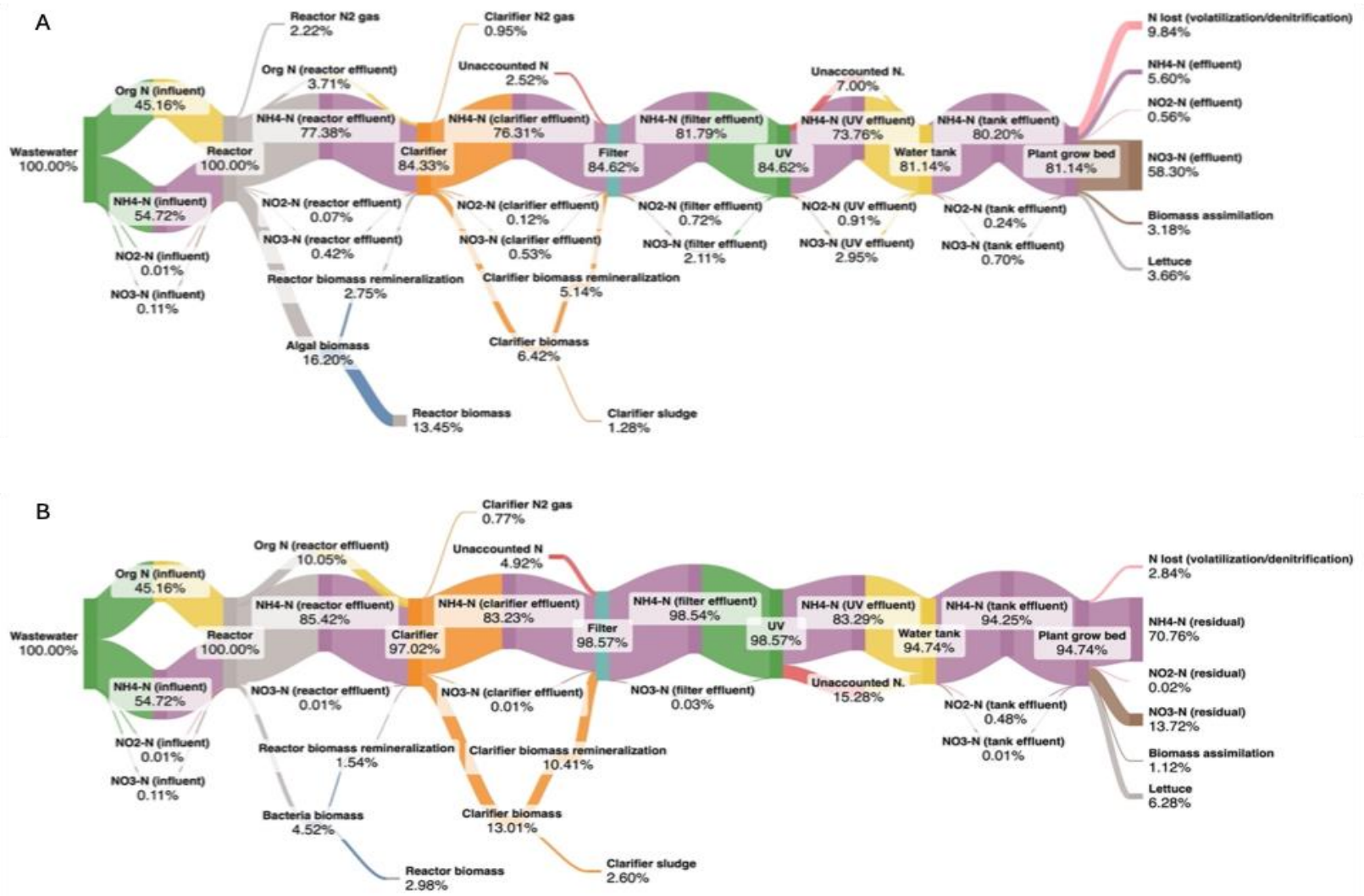


Figure 3.3: Flow of nitrogen through the algal-bacterial (A) and bacterial (B) treatment train in Poultryponics based on N mass basis from model parametrized with Trial 2 data. Unaccounted N was attributed to water loss (leakage) and volatilization in the system

In the bioreactors, robust mineralization of organic nitrogen and minimal nitrification resulted in NH_4-N remaining the dominant nitrogen form in the reactor effluents (77.4% in algal and 85.4% in bacterial system). Illumination of the algal bioreactors promoted algae growth, resulting in a biomass-assimilated fraction of N reaching 13.5%, compared to 3.0% in the non-illuminated bacterial bioreactors. Minimal denitrification (0.95% in the algal system and 0.77% in the bacterial system) and microbial biomass assimilation in the clarifiers (1.3 – 2.6% in both systems) resulted in continued nitrogen retention. NH_4-N remained the dominant nitrogen form through the clarifiers, filters, and UV disinfection, with 73.8% and 83.3% of N as NH_4-N in the UV effluent from the algal and bacterial systems, respectively (Figure **3.3**). The net unaccounted nitrogen was ~3.5% in the algal and ~3.3% in the bacterial system. This fraction of unaccounted nitrogen is likely due to water leakage or measurement error.

Additionally, microbial biomass retained in the bioreactors and clarifiers accounted for 17.9% and 6.7% of nitrogen losses in the algal and bacterial systems, respectively. This biomass has valuable applications in biofertilizers, biogas, bioplastics and animal feed production, contributing to nutrient recycling and nitrogen neutrality [126]. Furthermore, 13.0% and 3.6% of nitrogen was lost to denitrification in the algal and bacterial systems, respectively. Most of this loss occurred in the plant grow beds where nitrification was most robust. Higher rates of nitrification in the algal grow beds corresponded to the higher levels of denitrification observed in this system. Denitrification converts reactive nitrogen into N_2 gas, which is preferable to releasing NH_3 or N_2O into the environment [101]. However, without direct gas measurements in this study, it is unclear whether all nitrogen was reduced to N_2 or if NH_3 and N_2O, were also released. Future research is needed to confirm gas speciation and assess the potential global warming impact of nitrogen transformations in Poultryponics.

3.3.9 Nitrogen transformation in the hydroponic reservoirs and plant uptake

Nitrogen transformation in the hydroponic reservoirs differed between the algal and bacterial systems. Strong nitrification occurring in the algal system resulted in higher NO_3-N (58.3%) and lower NH_4-N (5.6%) (Figure **3.3**) in the effluent compared to the bacterial system (13.7% NO_3-N and 70.8% NH_4-N, Figure **3.3**B). This difference in nitrogen speciation was previously attributed to the higher pH (~7.5) maintained in algal reservoirs compared to the bacterial system's reservoirs (pH ~5.8) which suppressed nitrification [100].

Despite differences in nitrogen forms between the two systems, the bacterial system demonstrated a higher nitrogen uptake (6.3%) compared to the algal system, where only 3.7% of the nitrogen was assimilated into lettuce biomass (Figure **3.3**). Lettuce cultivated in the bacterial system achieved greater total fresh weight (121 g) and fresh head weight (91 g) compared to the algal system (108 g TFW and 59 g FHW), indicating more robust growth and higher nitrogen uptake demand. Overall, the non-supplemented Poultryponics system yielded between 1407 and 1895 g per 18 lettuce plants, with an overall nitrogen utilization efficiency (NUE) of 3.7% – 6.3%. This NUE is significantly lower than the 35 – 40% reported in other bioponic systems [25,65]. This outcome can largely be explained by two key factors: an undersized lettuce production system coupled with a continuous flow-through design. *A priori*, it was not clear whether sufficient nutrients would persist through treatment to reach the lettuce grow beds and so a conservatively sized system was designed. However, this design ensured consistent wastewater treatment but also led to nitrogen inputs exceeding plant demands, even though nitrogen concentrations were relatively low in PPW (Table **3.3**). In contrast, previous bioponic studies typically employed recirculating systems with minimal nitrogen input (4 – 10 g N per harvesting cycle of 35 days with

18 lettuce plants) and negligible nitrogen loss, allowing the plants to gradually access nitrogen over time, which enhances NUE [61,127].

The nitrogen mass balance from Trial 2 confirms that nitrogen was not a limiting factor for lettuce growth. Substantial nitrogen fractions remained in the reservoir effluent, particularly in the form of NH_4-N (5.6% in the algal system and 70.8% in the bacterial system) and NO_3-N (58.3% in the algal system and 13.7% in the bacterial system). These results highlight a mismatch between nitrogen supply (total N input = ~213 g per harvesting cycle of 35 days) and plant demand.

The substantial residual nitrogen concentrations in the effluent from both systems suggest the potential for recycling the effluent for additional crop production in a much larger hydroponic system. With the current NUE ranging from 3.7% to 6.3%, the nitrogen input of ~213 g could theoretically support the production of up to 30 – 38 kg of lettuce. Nevertheless, as other nutrients (K^+, Mg^{2+}, Ca^{2+} and Cu^{2+}) were found to limit lettuce growth, nutrient supplementation would be essential to prevent deficiencies and support optimal plant growth, similar to the findings of others [127]. Integrating partial recirculation with targeted nutrient dosing could improve nutrient uptake and enhance NUE while maintaining efficient wastewater treatment.

3.4 Conclusion

This study demonstrates that lettuce cultivated solely on treated poultry processing wastewater (PPW) exhibited 58±10% lower shoot growth ($p < 0.05$), 5±1% higher root biomass allocation and nutrient deficiencies (K, Mg, Ca, and Cu) compared to mineral fertilizer treatments. pH control (pH=7.0) and nutrient supplementation (N, P, K and micronutrients) alleviated growth inhibition, improving physiological metrics to levels comparable to the mineral fertilizer treatments. Low nitrogen utilization efficiencies (NUE = 3.7% – 6.3%) indicated nitrogen was not limiting, suggesting potential for expanding the hydroponic system. These findings demonstrate the value of treated wastewater in reducing reliance on synthetic fertilizers, promoting nutrient circularity, and supporting sustainable agricultural practices.

Chapter 4: Dosing *Salmonella* into Poultryponics: Fate of *Salmonella* during treatment of poultry processing wastewater and irrigation of hydroponic lettuce

Dosing *Salmonella* into Poultryponics: Fate of *Salmonella* during treatment of poultry processing wastewater and irrigation of hydroponic lettuce

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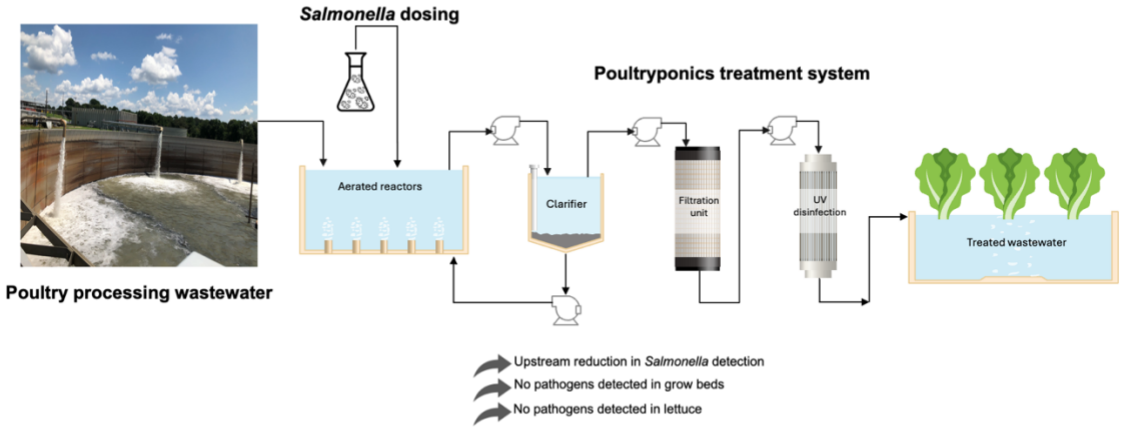
Abstract

"Poultryponics" is a bioponics system that treats poultry processing wastewater (PPW) for hydroponic irrigation. Effective pathogen removal is an under-studied barrier to advancing bioponics technology. This study evaluated the system's capacity to manage high exogenous doses of three *Salmonella* serotypes commonly associated with poultry. The Poultryponics treatment train, operating at ~115 liters d^{-1}, consisted of bioreactors (algal vs. bacterial), clarifiers, membrane filters, UV disinfection, water storage reservoir, and hydroponic grow beds. The fate of *Salmonella* sp. in the system was evaluated by analyzing water and lettuce samples for *Salmonella* contamination across three experimental stages: pre-inoculation (days 0-10), inoculation (days 11-20), and recovery (days 21-30). During the inoculation phase, bioreactors were spiked daily at levels significantly higher than those typically detected in PPW (<1 $\log_{10}$ CFU mL^{-1} *Salmonella*). When challenged at 3 $\log_{10}$ CFU mL^{-1}, the *Salmonella* levels observed in the algal and bacterial bioreactors were 0.6 and 1.4 $\log_{10}$ CFU mL^{-1}, representing a 99.6% and 97.5% reduction, respectively (p = 0.03). UV disinfection eliminated *Salmonella* from water samples, with no *Salmonella* detection in lettuce. At the 5 $\log_{10}$ CFU mL^{-1} dosage, *Salmonella* counts in bioreactor effluent were reduced to approximately 3 $\log_{10}$ CFU mL^{-1} by day 15 of inoculation (99% reduction). However, as *Salmonella* dosage continued, removal efficiency decreased, with *Salmonella* detection after UV treatment but not in grow beds or lettuce. This study confirms the effectiveness of the Poultryponics system in managing *Salmonella* under extreme contamination scenarios.

Keywords: Bioponics, Food pathogen, Hydroponics, Lettuce; Wastewater treatment

Graphical abstract



Highlights

1. Poultry processing wastewater has low background pathogen levels
2. No culturable *Salmonella* was detected in grow beds at 10^3 CFU mL^{-1} dosage
3. Potential *Salmonella* reactivation was detected at 10^5 CFU mL^{-1}
4. No detection of pathogens in lettuce

4.1 Introduction

Access to clean freshwater is increasingly constrained in the agricultural sector, compelling the exploration of sustainable and alternative water sources for food production [128]. This shift has necessitated the development of technologies like bioponics, which integrates biological wastewater treatment with soilless agriculture (hydroponics) [20,25,60,62,63]. Bioponic systems reduce environmental nutrient pollution by offering a sustainable pathway to utilize nutrient-rich wastewater for the irrigation of a variety of edible crops including lettuce, beets, broccoli, and tomatoes [27,64].

In the United States, where the poultry industry is both a major agricultural sector and a significant contributor to wastewater production (~250 billion liters in 2021) [5,8,54], the potential for reusing poultry processing wastewater (PPW) is immense. Although PPW contains high levels of plant nutrients [10], poultry processing facilities typically discharge their wastewater to municipal systems or discharge directly into the environment after onsite treatment [8]. Such systems aim to eliminate nutrients rather than re-use them. Our team has developed the "Poultryponics" concept, integrating PPW treatment with hydroponic agriculture to create a resource-efficient, closed-loop system [100]. To our knowledge, it is the only continuous pilot system that treats poultry processing wastewater for irrigation of ready-to-eat vegetables.

However, PPW contains various foodborne pathogens including *Salmonella*, *Escherichia coli* (*E. coli*), and *Campylobacter* [13–15], which raises significant food safety concerns when used for irrigating ready-to-eat vegetables such as lettuce, given the prevalence of bacterial foodborne outbreaks in the U.S. [16,17,129]. The Centers for Disease Control and Prevention (CDC) estimates approximately 1.4 million foodborne infections and 420 deaths linked to *Salmonella* [18], with poultry meat identified as one of the major sources of the bacteria. Furthermore, our recent survey

of PPW from commercial processing plants across the southeastern U.S., 50% (4 of 8) of processing facilities tested positive for several strains of nontyphoidal *Salmonella* enterica (Table **4.1**), albeit at low concentrations (<1 log $_{10}$ CFU mL $^{-1}$). Despite these low concentrations, *Salmonella* presence in wastewater used for hydroponic irrigation can pose significant public health risks due to the potential for pathogen persistence [130,131].

Table 4.1: *Salmonella* serotypes isolation from PPW samples across multiple processing facilities in southeastern U.S.

Plant	Sampling point	Serotypes
Plant 2	Inside DAF 1	Infantis
	Inside DAF 2	Infantis, Kentucky
	Final effluent	Enteritidis, Kentucky
Plant 3	Inside DAF 1	Newport
	Final effluent	Rough_O:r:1,5
Plant 6	DAF 1 effluent	Kentucky
	DAF 2 effluent	Hadar
Plant 8	DAF 1 effluent	Hadar

DAF: Dissolved air floatation unit

Therefore, successful implementation of wastewater reuse for food crop irrigation hinges on ensuring effective pathogen removal. Past research on treatment of poultry processing wastewater for pathogen removal has generally relied on advanced technologies such as ultrafiltration and reverse osmosis [37,132]. While these approaches are effective in reducing microbial loads, their high cost and energy requirements could limit their practical application in agricultural operations [133]. Our pilot-scale Poultryponics study, which was carried out over 220 days of continuous operation [100], demonstrated that pathogens in PPW (*Salmonella*, *E. coli*, and

Campylobacter) were eliminated in the aerated bioreactors [100], highlighting the potential of simpler, cost-effective biological treatment. This was primarily attributed to competition with background heterotrophic bacteria [134] and high dissolved oxygen concentration [42,135]. Subsequent clarification, simple filtration (5 μm bag filters), and UV disinfection in the treatment system resulted in further bacterial reductions (~2 log reduction of total aerobic bacteria) with no detection of pathogens in hydroponic lettuce [100].

However, the overall system's ability to remove food pathogens could not be effectively observed because no pathogens were detectable after the first treatment step (aerated bioreactors), even with enrichment. Additionally, a majority of the raw wastewater samples collected also tested negative for *Salmonella* in our previous trials [100]. The low background concentration of *Salmonella* in PPW creates a significant knowledge gap in evaluating the system's efficacy under short-term high pathogen loads, particularly in scenarios where pathogen concentrations far exceed typical levels observed in PPW. Such conditions, which could arise from upstream disinfection failures or contamination events, represent critical worst-case scenarios that bioponic systems must address.

Furthermore, current bioponic studies do not explore pathogen dynamics and food safety [24,127,136], as several studies often rely on sterile chemical solutions (artificial wastewaters) rather than real wastewater [69,70,137]. This practice lacks real-world applicability, failing to address the impacts of foodborne pathogens and food safety during hydroponic irrigation with real wastewater. *Salmonella* populations have been reported to persist for over 336 days in surface water [138] and may therefore survive and proliferate in wastewaters and hydroponic systems even at low concentrations, posing risks to food safety and public health [139,140].

This gap underscores the necessity to better understand and mitigate the risks associated with foodborne pathogens using bioponic systems for crop production. The objective of this study was to evaluate the fate of high *Salmonella* influx in a pilot-scale treatment system utilizing real poultry processing wastewater for lettuce production. The exogenous pathogen spiking was conducted to simulate a "worst-case scenario", testing the Poultryponics system's capability to manage a sudden influx of pathogens from a poultry processing plant. The high influxes of *Salmonella* also shed light on the effectiveness of different operations in the treatment train at removing these pathogens.

4.2 Materials and methods

4.2.1 Pilot-scale Poultryponics treatment system

A comprehensive description and design of the Poultryponics treatment system is provided in our previous study [100]. Briefly, the pilot-scale treatment system was set up to treat ~115 L d^{-1} of PPW split between two parallel treatment trains: an algal-bacteria system (Treatment system 1) and a bacterial system (Treatment system 2). Poultry processing wastewater was collected regularly (~every 10 days) from the effluent of the dissolved air floatation (DAF) unit at a commercial broiler processing facility and continuously pumped into the treatment system. Each treatment train was composed of bioreactors (3 replicates), clarifiers, 1-micron bag filters to reduce solids, UV disinfection, water holding tank, and a deep-water system for lettuce cultivation (Figure **4.1**). Notably, this system does not make use of costly ultrafiltration or reverse osmosis systems but relies instead on biological treatment and physical treatments similar to a wastewater treatment plant. Both treatment trains were the same except for the bioreactors, where algae growth was promoted in Treatment system 1 with illumination using LED lights and suppressed in Treatment system 2 by covering the bioreactors. Butterhead lettuce was grown in 3 replicate grow beds (~38 L each) per treatment train with six heads of lettuce in each grow bed. Another set of 3 grow beds with 6 lettuce heads each were filled with a modified Sonneveld's solution [73] as a control (Treatment 3).

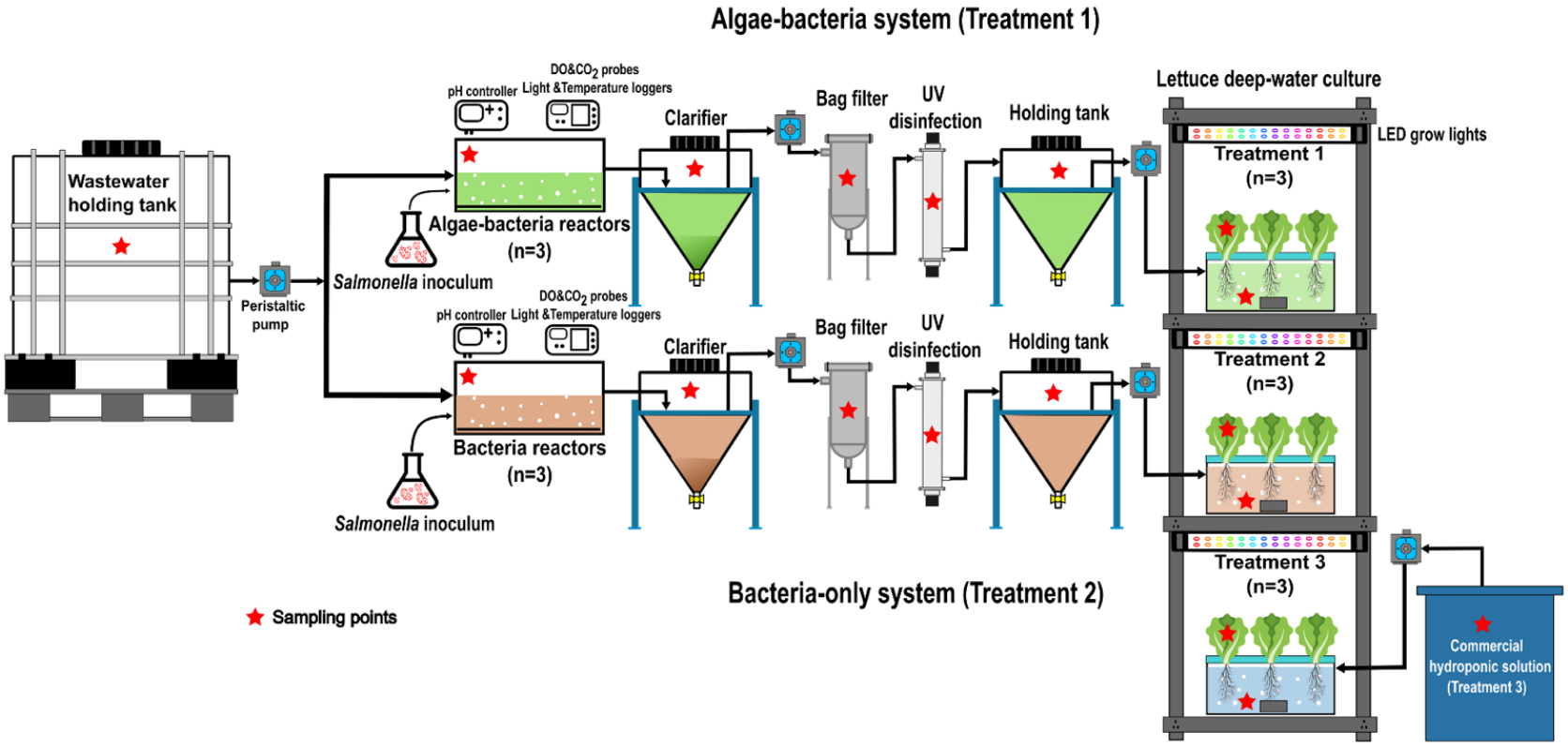


Figure 4.1: Schematic representation of the pilot-scale Poultryponics system. Treatments 1 and 2 irrigated lettuce with effluents from the algal-bacteria and bacterial treatment train respectively. Sonneveld's solution was used as control (Treatment 3)

4.2.2 Experimental design

The experimental design followed three distinct phases to evaluate the system's response to *Salmonella* influx and its subsequent recovery (Figure **4.2**). In the pre-inoculation phase (days 0–10 of lettuce production), the system operated without any exogenous *Salmonella* dosing, such that it operated with background pathogen levels typically detected in the poultry processing wastewater. After this stage, water samples and two lettuce heads from each grow bed (n = 6 per treatment) were destructively harvested and analyzed for pathogens on day 10. The inoculation phase (days 11-20 of lettuce production) involved spiking in high doses of *Salmonella* into the bioreactors daily, with concentrations of 3 and 5 $\log_{10}$ CFU mL^{-1} in experiments 1 and 2 respectively. These *Salmonella* dosages were chosen to simulate extreme contamination scenarios, representing several orders of magnitude higher than the levels typically detected in dissolved air flotation (DAF)-treated PPW, which are typically below 1 $\log_{10}$ CFU mL^{-1} and detected only through selective enrichment [100]. This low baseline is consistent with *Salmonella* concentrations reported for pre-processing and pre-chill broiler carcasses, where the prevalence is detected predominantly via enrichment methods [141,142]. By selecting dosages near the total coliform count in PPW, this study aimed to assess the system's performance under a worst-case-scenario threshold of *Salmonella* contamination, mimicking severe upstream disinfection failure or contamination event. The 5 $\log_{10}$ CFU mL^{-1} was tested partly in response to the results of the experiment with 3 $\log_{10}$ CFU mL^{-1} in an effort to push the system to its limits.

Approximately 8 h post-inoculation, water samples were collected from various sampling points in the treatment train for *Salmonella* enumeration. On day 20, both water and lettuce samples were analyzed for pathogens. During the recovery phase (days 21-30 of lettuce

production), *Salmonella* spiking ceased, allowing the system to return to baseline influent pathogen levels. Water and remaining lettuce samples were analyzed for pathogens on day 31.

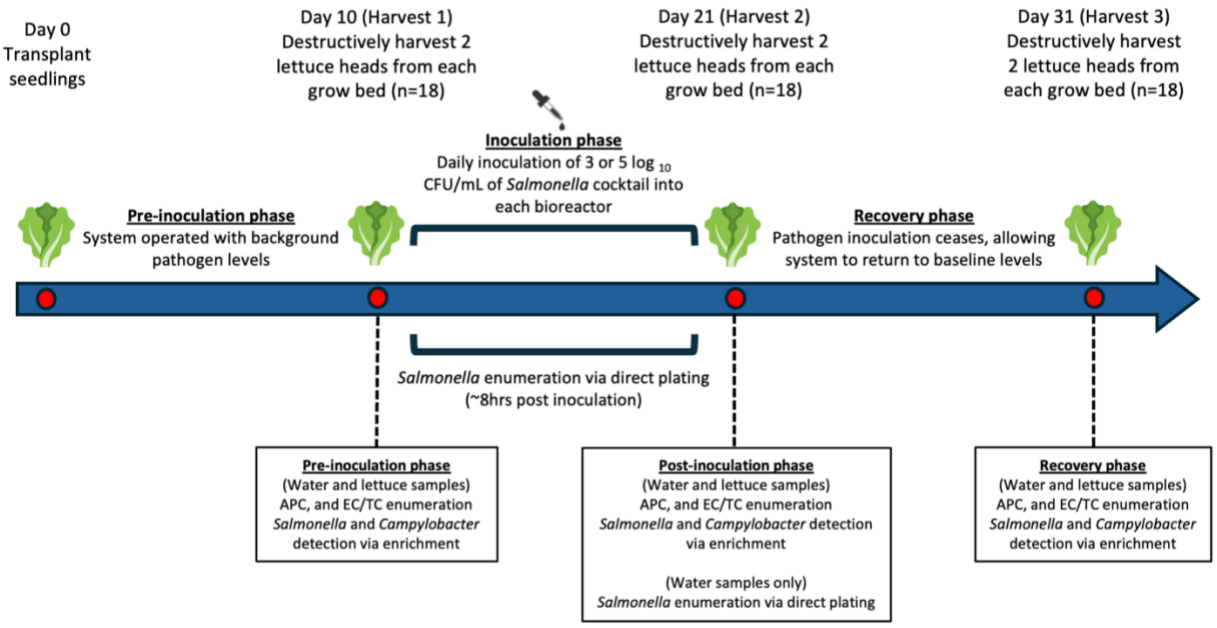


Figure 4.2: Experimental timeline to assess the system's response to Salmonella influx. The study followed a structured approach over three phases: pre-inoculation (days 0-10); inoculation (days 11-20): where Salmonella was introduced at high doses; and recovery (days 21-30). Water and lettuce samples were analyzed at each stage to monitor pathogen dynamics.

4.2.3 Preparation of *Salmonella* seed culture

In this study, three serotypes of *Salmonella enterica*: *S.* Enteritidis, *S.* Infantis, and *S.* Typhimurium, were selected for the inoculation experiments. These serotypes were chosen due to their prevalence in poultry and their significance as zoonotic pathogens in humans [143]. Glycerol

stocks of each serotype, stored at -80°C, were retrieved for initial culture preparation. Each strain was incubated for 24 h at 37°C on Xylose Lysine Tergitol 4 (XLT4) agar, after which a single colony was transferred onto Standard Methods Agar (SMA) and incubated for another 24 h at 37°C. On the morning of the experiment, colonies were scraped from the SMA plates and suspended in sterile phosphate-buffered saline (PBS) to produce a cell suspension with a concentration of 10^8 CFU mL $^{-1}$, as determined by its optical density of 0.15 when measured at an absorbance of 540 nm [144,145]. To investigate the combined effect of these serotypes and assess their survivability and competitive growth dynamics in the system, the inoculum of the three *Salmonella* serotypes was mixed in equal volumes. The *Salmonella* cocktail was then dosed into the bioreactors to achieve the desired inoculum concentrations of 10^3 and 10^5 CFU mL $^{-1}$ by adding either 190 µL or 19 mL of inoculum respectively to each bioreactor daily (19 L working volume per bioreactor).

4.2.4 Microbiological assessment

Water samples were serially diluted in 9 g L^{-1} sterile saline water and 1 mL of selected dilution was plated in duplicate on respective petrifilms (3M, USA) for counts of general indicators: aerobic plate counts (APC), *Escherichia coli* (*E. coli*) and total coliforms (TC). For *Salmonella* enumeration, 0.1 mL of selected dilutions were plated in duplicate on XLT4 agar. Water samples without counts (presumptive negative) were enriched by adding 25 mL of water sample to 25 mL 2X buffered peptone water (ThermoFisher) and incubated for 24 h at 37 °C. The pre-enriched samples were inoculated into Rappaport Vassiliadis (Hardy Diagnostics, USA) broth, and Tetrathionate broth supplemented with iodine solution (Hardy Diagnostics, USA), incubated for 24 h at 42 °C and plated in duplicate on XLT4. Presumptive *Salmonella* isolates were

confirmed with the 3M Molecular Detection System (MDS). Rapid plate agglutination test was performed on the *Salmonella* isolates by adding one drop (~20 μL) of *Salmonella* O antiserum (Hardy Diagnostics, USA) onto a clean glass slide, mixing with a loopful of isolated colonies and observing the results within one minute. For *Campylobacter* detection, 25 mL of water was added to 25 mL 2X Bolton broth and incubated under microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2) for 48 h at 42 °C [75]. The resultant enrichment was plated in duplicate on Campy Cefex agar (Neogen, Michigan, USA) and incubated under microaerobic conditions for 48 h at 42 °C.

Methods for enumeration of microbial profile in lettuce were performed based on a previous study [146] with minor modifications. A full lettuce head was homogenized with a sterilized hand-held homogenizer (Pro Scientific Inc), and 5 g of lettuce homogenate was added to 45 mL 1X buffered peptone water. Samples were vortexed, serially diluted with 9 g L^{-1} sterile saline water, and plated in duplicates on respective petrifilms for APC, *E. coli*, and other coliforms. The method used for *Salmonella* isolation followed USDA/FSIS/Bacteriological Analytical Manual's *Preparation of foods for isolation of Salmonella from Fresh leafy green vegetables, herbs, and sprouts* [147]. Briefly, 5 g of lettuce homogenate was added to 45 mL universal pre-enrichment broth, and incubated for 24 h at 37°C. The pre-enriched broth was inoculated into Rappaport Vassiliadis and Tetrathionate broths, incubated for 24 h at 42 °C and plated in duplicate on XLT4. Presumptive *Salmonella* isolates were confirmed as described above. Detection of *Campylobacter* was performed by enriching 5 g of lettuce homogenate in 45 mL of 1X Bolton broth, incubated for 48 h at 42°C, and plated onto Campy Cefex agar as described above.

4.2.5 Mass balance model

A mass balance model that assumes no pathogen removal or inactivation (dilution effects only) of the system was developed. This was necessary because *Salmonella* was pulsed in once per day, not continuously fed in the influent wastewater. Each process unit within the system was modeled for predicted pathogen concentration if no inactivation/ removal occurs by using Equation 4. This model assumes each process in the system is well mixed, which is a conservative assumption given that the model is compared against observed *Salmonella* concentrations. Equation 4 was employed for each operation in the treatment train and thus accounts for dilution effects that are expected to occur as the pulse of *Salmonella* moves through the system.

$$\mathbf{C_i = C_{i-1} + F/V\ (C_f - C_{i-1})\Delta t} \qquad \text{Equation 4}$$

where:

C_i = predicted concentration of *Salmonella* in effluent of current operation

C_{i-1} = concentration of *Salmonella* in effluent of current operation at previous time step

F = flow rate of water through operation

V = working volume of operation

C_f = concentration of *Salmonella* in feed stream to this operation (effluent of previous operation)

Δt = time step of 10 minutes

4.2.6 Statistical analyses

All counts were transformed to $\log_{10}$ CFU mL $^{-1}$ and $\log_{10}$ CFU g^{-1} fresh weight for water and lettuce samples respectively. The differences in TC and APC between the bioreactors in the two treatment trains were analyzed in Microsoft Excel using a paired *t* test with significance at a *p*-value ≤ 0.05. The measured *Salmonella* concentrations in the bioreactors during the inoculation phase were compared to the mass balance model. For the microbial loads in lettuce, the means and standard deviations were compared using analysis of variance and Tukey's test in R (v. 4.4.0) using the "multcomp" package.

4.3 Results and discussion

4.3.1 Background microbial characteristics of poultry processing wastewater

The microbial characteristics of the 8 wastewater batches that were collected from the processing plant are summarized in Table **4.2**. The aerobic plate counts from the water samples ranged from 5.4 to 7.1 $\log_{10}$ CFU mL $^{-1}$, indicating the presence of aerobic microorganisms. Total coliforms ranged from 3.9 to 6.0 $\log_{10}$ CFU mL $^{-1}$. Approximately 1 $\log_{10}$ CFU mL $^{-1}$ of *E. coli* was detected in only 2 samples while *Campylobacter* was not detected in any wastewater samples collected. Throughout this study, *E. coli* and *Campylobacter* were not detected in any of the process units in the Poultryponics system. *Salmonella* presence was detected in 2 out of 8 wastewater batches with enrichments, indicating low starting concentrations (<1 $\log_{10}$ CFU mL $^{-1}$) in PPW. The notably low concentrations of *E. coli*, *Campylobacter,* and *Salmonella* in PPW could be attributed partly to the efficacy of antimicrobial agents such as peracetic acid (PAA) and cetylpyridinium chloride (CPC) used in postchill decontamination [148]. However, these results are consistent with previous reports on the microbial loads of DAF effluents collected regularly from the same processing facility over a period of 9 months [100]. Furthermore, it establishes baseline pathogen levels in PPW and provides a basis for the pathogen challenge experiments reported here.

4.3.2 Treatment performance under 10^3 CFU mL $^{-1}$ *Salmonella* dosage

There was no detection of *Salmonella* in all water samples collected from various sampling points in the treatment system during the pre-inoculation phase (Figure **4.3**). The wastewater tank, bioreactors, and clarifiers exhibited high aerobic bacteria counts, ranging from 4.3 to 5.5 $\log_{10}$ CFU mL $^{-1}$. The increase in APC was due to microbial biomass growth, which is essential for organics removal, nutrient mineralization and transformation in PPW. These findings are

consistent with our previous report, which demonstrated robust heterotrophic activity in the wastewater tank and bioreactors resulting in high soluble chemical oxygen demand (sCOD) removal efficiencies (>80%) [100]. Although *Salmonella* was sporadically detected in PPW (Table **4.2**), its absence in the pre-inoculation phase could be attributed to it being outcompeted by other heterotrophic bacteria in the wastewater tank, bioreactors, and clarifiers [100]. The decline in *Salmonella* populations in environmental water samples has been attributed to a combination of biotic factors such as unsuccessful nutrient competition with background microbes [134], protozoal grazing [149], and bacteriophage lysis [150]. Moreover, while *E. coli* was not detected in the pre-inoculation phase, total coliform counts in water samples ranged between 2 and 3 $\log_{10}$ CFU mL $^{-1}$. However, UV disinfection achieved approximately 1 log reduction of total coliforms. Although the clarifiers and 1 μm filters improved water clarity in preparation for UV disinfection, they provided no measurable reduction in total coliforms or APC (Figure **4.3**)

Table 4.2: Summary of microbial loads in batches of poultry processing wastewater

	Date of collection	$\log_{10}$ CFU mL $^{-1}$			*Salmonella*	*Campylobacter*
		E. coli	Total coliforms	Aerobic plate count		
Experiment 1	8/17/23	1.11	3.89	5.44	nd	nd
	8/23/23	1.37	4.79	7.01	+	nd
	9/8/23	0	5.97	7.06	nd	nd
	9/19/23	0	3.90	5.87	nd	nd
	9/28/23	0	3.74	5.77	nd	nd
Experiment 2	10/23/23	0	4.58	5.72	nd	nd
	11/3/23	0	4.12	6.78	+	nd
	11/15/23	0	4.79	6.66	nd	nd

+ : detected after enrichment; nd: not detected after enrichment

Sampling point	log $_{10}$ CFU/mL								
	Pre-inoculation phase (Day 10)			Post-inoculation phase (Day 21)			Recovery phase (Day 31)		
	Total coliforms	Aerobic Plate Count	*Salmonella*	Total coliforms	Aerobic Plate Count	*Salmonella*	Total coliforms	Aerobic Plate Count	*Salmonella*
Wastewater Tank	3.40	4.83	-	3.84	4.91	+	3.40	4.43	-
Algal bioreactors (n = 3)	2.06 ± 0.28^{b}	4.33 ± 0.18^{a}	-	2.53 ± 0.34^{b}	4.40 ± 0.46^{a}	+	2.54 ± 0.29^{b}	4.26 ± 0.20^{a}	-
Bacterial bioreactors (n = 3)	2.53 ± 0.77^{b}	4.71 ± 0.46^{a}	-	2.52 ± 0.56^{b}	4.22 ± 0.43^{a}	+	2.72 ± 0.25^{b}	4.91 ± 0.66^{a}	-
Algal clarifier	2.62	4.40	-	3.01	5.48	+	2.94	4.50	-
Bacterial clarifier	3.08	5.45	-	3.08	5.54	+	2.91	5.20	-
Algal filter	2.74	4.02	-	2.97	5.34	+	2.74	4.89	-
Bacterial filter	2.79	5.13	-	3.04	5.10	+	2.79	5.11	-
Algal UV	2.35	3.88	-	2.00	3.38	-	2.00	4.12	-
Bacterial UV	1.71	3.89	-	1.48	3.62	-	1.94	4.13	-
Holding tank (Algal)	2.34	5.88	-	3.09	5.08	-	3.13	6.03	-
Holding tank (Bacterial)	3.63	5.97	-	3.53	6.74	-	3.89	5.60	-
Holding tank (Control)	3.80	5.77	-	2.26	3.32	-	2.87	4.19	-

scale: 7, 6, 5, 4, 3, 2, 1, 0

Figure 4.3: Enumeration of bacteria and Salmonella detection in the treatment system before, during, and after 10^3 CFU mL $^{-1}$ Salmonella inoculation. Values in bioreactors are the means ± SD of three biological replicates (n = 3). Within each phase, means with similar letters are not statistically different. + and – represent presence and non-detection of Salmonella respectively.

During the 10-day *Salmonella* inoculation phase with 3 $\log_{10}$ CFU mL^{-1}, direct plating mostly yielded no viable counts for *Salmonella* until day 20 (Figure **4.4**). The observed levels of *Salmonella* (< 2 $\log_{10}$ CFU mL $^{-1}$) across all treatment stages were at least 1 $\log_{10}$ CFU mL $^{-1}$ lower than what is predicted by the mass balance model of the system (Figure **4.5**A), indicating robust removal. In this experiment, the viable *Salmonella* counts on day 20 in the algal bioreactors (~0.6 $\log_{10}$ CFU mL $^{-1}$, 99.6% reduction) were significantly lower (p = 0.03) than those in the bacterial bioreactors (~1.4 $\log_{10}$ CFU mL $^{-1}$, 97.5% reduction). Bhatt et al. [41] reported 94 – 97% reduction of *Salmonella* sp. in post-primary and post-secondary municipal wastewater, with similar *Salmonella* concentrations (3.9 – 4.6 $\log_{10}$ CFU mL $^{-1}$), that was treated with algae and attributed the effect to photooxidation in algal reactors. The observed *Salmonella* reduction in this study

could similarly be influenced by photooxidative effects driven by algal photosynthesis in the bioreactors. The algal bioreactors were illuminated and sparged with CO_2, which spurred algae growth, resulting in a total suspended solids (TSS) concentration of ~9 g L^{-1} compared to ~2.5 g L^{-1} in the bacterial bioreactors which lacked illumination and CO_2 supplementation, and consequently did not support algae growth and photosynthesis [100]. Algal photosynthesis generates reactive oxygen species which have been shown to effectively inactivate pathogens [42,151,152]. Additionally, algal exudates contain bioactive compounds (such as phenols and fatty acids) that possess antimicrobial properties against different species of pathogenic bacteria [153].

Sampling point	10^3 CFU/mL *Salmonella* dosing					
	Day 11	Day 13	Day 15	Day 18	Day 20	Day 21
Algal bioreactors (n = 3)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.57 ± 0.51	0 ± 0
Bacterial bioreactors (n = 3)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.42 ± 0.23	0 ± 0
Algal clarifier	0.00	0.00	0.00	0.00	1.18	0.00
Bacterial clarifier	1.30	0.00	0.00	0.00	2.00	0.00
Algal filter	0.00	0.00	0.00	0.00	1.00	0.70
Bacterial filter	1.70	0.00	0.00	0.00	1.90	0.70
Algal UV	0.00	0.00	0.00	0.00	0.00	0.00
Bacterial UV	0.00	0.00	0.00	0.00	0.00	0.00
Holding tank (Algal)	0.00	0.00	0.00	0.00	0.00	0.00
Holding tank (Bacterial)	0.00	0.00	0.00	0.00	0.00	0.00
Sampling point	10^5 CFU/mL *Salmonella* dosing					
	Day 11	Day 13	Day 15	Day 18	Day 20	Day 21
Algal bioreactors (n = 3)	3.41 ± 0.65	3.71 ± 1.03	2.97 ± 0.03	4.17 ± 0.71	4.34 ± 0.64	2.71 ± 0.91
Bacterial bioreactors (n = 3)	4.44 ± 0.89	4.46 ± 0.52	2.53 ± 0.64	4.42 ± 0.65	3.91 ± 1.31	3.31 ± 1.16
Algal clarifier	4.17	4.69	3.23	4.48	4.71	3.61
Bacterial clarifier	4.11	4.09	2.62	4.32	4.81	3.91
Algal filter	3.73	4.16	3.25	4.39	4.62	3.70
Bacterial filter	3.88	4.20	2.30	4.30	4.62	4.16
Algal UV	0.00	0.00	0.00	0.00	0.00	0.00
Bacterial UV	0.00	0.00	0.00	0.00	0.00	0.00
Holding tank (Algal)	0.00	0.00	0.00	0.00	0.00	0.00
Holding tank (Bacterial)	0.00	0.00	0.00	0.00	0.00	0.00

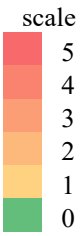


Figure 4.4: Enumeration of Salmonella in the treatment system during the inoculation phase. Data in the figure is expressed as $\log_{10}$ CFU mL^{-1}. Values in bioreactors are the means ± SD of three biological replicates (n = 3).

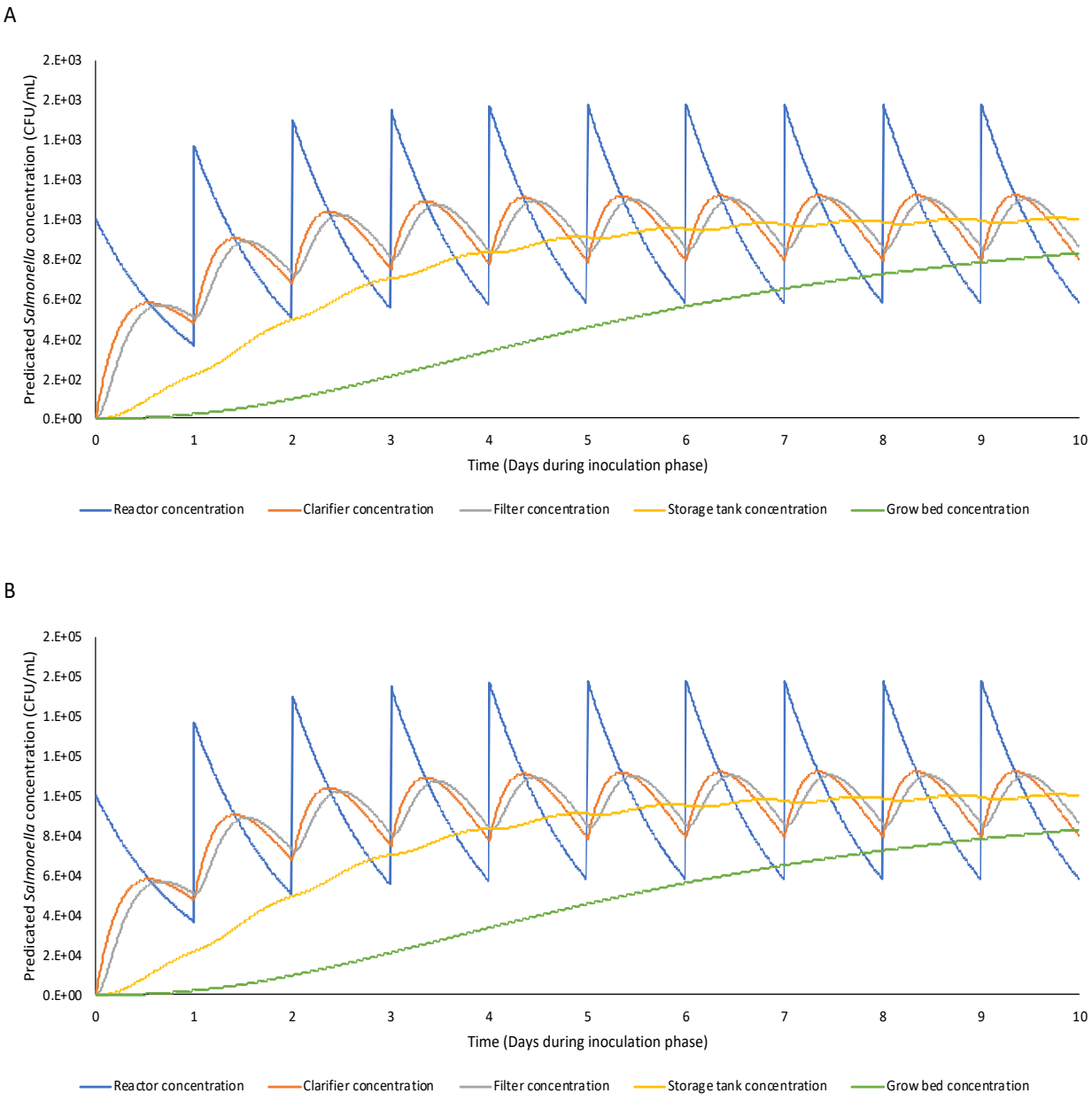


Figure 4.5: *Salmonella* concentrations as predicated by a mass balance model that assumes no removal or inactivation. Changes in concentration over time are the result of dilution effects following daily pathogen spikes at 10^3 CFU mL $^{-1}$ (A) or 10^5 CFU mL $^{-1}$ (B).

The lower levels of *Salmonella* in the algal system persisted into the post clarifier and post filtration stages during the 10^3 CFU mL $^{-1}$ experiment. On day 21, direct plating of water samples showed no *Salmonella* counts at most sampling points (Figure **4.4**); however, *Salmonella* was detectable via enrichment in all units prior to UV disinfection (Figure **4.3**). To ascertain if the observed reduction in viable *Salmonella* counts at 10^3 CFU mL $^{-1}$ dosage was a result of adherence/attachment to the algal-bacterial biomass within the treatment system, microbial sludge samples from bioreactors, clarifiers, and filters were collected and enriched for *Salmonella* presence. The analysis revealed that all solid samples tested positive for *Salmonella* (Table **4.3**), suggesting the potential adsorption of *Salmonella* into the algal-bacterial biomass within the treatment system [42]. Similar to the pre-inoculation phase, *Salmonella* was not detected in water samples downstream of UV disinfection: UV effluents, water holding tanks, or hydroponic grow beds (Figure **4.3**).

At the end of the recovery phase (day 31), aerobic plate counts and total coliforms in each treatment unit were similar to those of the pre-inoculation phase. Additionally, *Salmonella* was not detected in all water samples in the treatment system (Figure **4.3**), suggesting a full recovery. This indicates that the lifespan of *Salmonella* was short while it existed in the treatment system. Furthermore, *E. coli*, *Campylobacter,* and *Salmonella* were not detected in any of the lettuce samples cultivated on treated PPW dosed with 10^3 CFU mL $^{-1}$ *Salmonella* (Table **4.4**). However, the counts for aerobic bacteria (4.5 – 6.5 $\log_{10}$ CFU g^{-1}) and total coliforms (3.8 – 5.1 $\log_{10}$ CFU g^{-1}) were higher than previous studies on hydroponic and soil-grown romaine lettuce, which showed aerobic plate counts ranging from 3 to 4 $\log_{10}$ CFU g^{-1} and total coliforms ranging from 2 to 3.2 $\log_{10}$ CFU g^{-1} [146]. It is important to note that the lettuce samples analyzed in this study did not undergo any form of post-harvest treatment such as washing with antimicrobials [154], chlorine

[155], ozonized water [156] etc., unlike the lettuce samples examined by Mohammad et al. [146], which were obtained from retail stores and were likely decontaminated before being offered for sale.

Table 4.3: Isolation of *Salmonella* in solids collected from various units in the treatment system.

Sampling point	Enrichment broth with presumptive colonies on XLT4	MDS output
Algal reactor 1	BPW, RV, TT	+ (in all broths)
Algal reactor 2	BPW, RV, TT	+ (in all broths)
Algal reactor 3	BPW, RV, TT	+ (in all broths)
Bacterial reactor 1	RV, TT	+ (in all broths)
Bacterial reactor 2	RV, TT	+ (in all broths)
Bacterial reactor 3	BPW, RV, TT	+ (in all broths)
Algal clarifier	RV, TT	+ (in all broths)
Bacterial clarifier	BPW, RV, TT	+ (in all broths)
Algal filter	RV, TT	+ (in all broths)
Bacterial filter	TT	+

BPW: Buffered peptone water; RV: Rappaport Vassiliadis broth; TT: Tetrathionate broth
MDS: 3M Molecular Detection System; +: detected after enrichment

Although administering 10^3 CFU mL $^{-1}$ *Salmonella* vastly exceeds the baseline microbial profile in PPW (Table **4.2**), this concentration did not sufficiently challenge the treatment system to fully assess its effectiveness at *Salmonella* reduction; non-detection of *Salmonella* occurred at various sampling points (Figure **4.4**). With direct plating carried out approximately 8 h post-inoculation, it was apparent that *Salmonella* did not thrive under the conditions within the Poultryponics system. The combination of bioreactor conditions such as competition with background heterotrophic bacteria, adsorption/sedimentation in biomass, high dissolved oxygen concentration (8.1 ± 4.1% air saturation), and slightly neutral pH (pH = 6.5) [100] resulted in negligible viable counts in the water samples.

Table 4.4: Bacterial enumeration and isolation of pathogens from lettuce samples cultivated during 10^3 CFU mL $^{-1}$ *Salmonella* inoculation.

Harvest	Lettuce	$\log_{10}$ CFU/g			*Salmonella*	*Campylobacter*
		E. coli	Total coliforms	Aerobic plate count		
1 (Day 10)	Algal system (n=6)	0 ± 0 [a]	4.72 ± 0.48 [a]	6.16 ± 0.48 [a]	nd	nd
	Bacterial system, (n=6)	0 ± 0 [a]	4.65 ± 0.40 [a]	5.44 ± 0.44 [b]	nd	nd
	Control (n=6)	0 ± 0 [a]	4.55 ± 0.46 [a]	5.69 ± 0.40 [ab]	nd	nd
2 (Day 21)	Algal system (n=6)	0 ± 0 [a]	4.42 ± 0.40 [a]	5.52 ± 0.61 [a]	nd	nd
	Bacterial system, (n=6)	0 ± 0 [a]	4.45 ± 0.37 [a]	5.42 ± 0.16 [a]	nd	nd
	Control (n=6)	0 ± 0 [a]	4.76 ± 0.54 [a]	5.87 ± 0.30 [a]	nd	nd
3 (Day 31)	Algal system (n=6)	0 ± 0 [a]	4.01 ± 0.24 [b]	5.01 ± 0.38 [a]	nd	nd
	Bacterial system, (n=6)	0 ± 0 [a]	4.30 ± 0.44 [ab]	4.96 ± 0.21 [a]	nd	nd
	Control (n=6)	0 ± 0 [a]	4.57 ± 0.12 [a]	5.41 ± 0.55 [a]	nd	nd

Values are the means ± SD of three biological replicates with two samples per replicate (n = 6). Within each harvest, means with similar letters are not statistically different based on the Tukey's test ($p>0.05$).
nd: not detected after enrichment

The full recovery of the system to baseline pathogen levels underscores the resilience of the system under moderate contamination loads. However, repeated pathogen exposure at higher dosages could potentially lead to shifts in microbial community structure, favoring resistant strains which may compromise food safety. This result led us to repeat the study but with an even higher dosage of *Salmonella* to better understand the system's treatment limits.

4.3.3 Treatment performance under 10^5 CFU mL $^{-1}$ *Salmonella* dosage

Similar to the first experiment, there was no detection of *Salmonella* at the end of the pre-inoculation phase (day 10) in all water samples (Figure **4.6**). Notably, aerobic plate counts (APC) and total coliform counts exhibited an increase of 1 and 2 $\log_{10}$ CFU mL $^{-1}$, respectively, across all bioreactors when compared to the earlier pre-inoculation phase at a 10^3 CFU mL $^{-1}$ *Salmonella* dosage (Figure **4.3**). This increase could be due to variability in microbial composition of the influent wastewater (Table **4.2**) or the dynamic nature of microbial communities in the bioreactors. However, subsequent stages in the treatment process resulted in reductions of aerobic bacteria and coliforms. Clarifiers, bag filters, and final UV disinfection, resulted in aerobic bacteria reductions of 0.2, 0.4, and 1.7 logs, respectively, culminating in bacterial concentrations in algal and bacterial water holding tanks similar to those of the pre-inoculation phase of experiment 1 (Figure **4.6**).

Despite the high daily influx of *Salmonella* at 10^5 CFU mL $^{-1}$, the algal bioreactors demonstrated a large suppression of culturable *Salmonella* counts, with an average log reduction of 1.4 (96.0%), in comparison to a 0.5 log (68.4%) reduction in the bacterial bioreactors between days 11 and 13 (Figure **4.4**). These results were generally consistent with the results from experiment 1 but with lower levels of reduction. By day 15, *Salmonella* counts had decreased to approximately 10^3 CFU mL $^{-1}$ (99.0% reduction) across all bioreactors, clarifiers, and filters.

Sampling point	log $_{10}$ CFU/mL								
	Pre-inoculation (Day 10)			Post-inoculation (Day 21)			Recovery phase (Day 31)		
	Total coliforms	Aerobic Plate Count	*Salmonella*	Total coliforms	Aerobic Plate Count	*Salmonella*	Total coliforms	Aerobic Plate Count	*Salmonella*
Wastewater Tank	6.04	6.10	-	5.61	5.97	+	4.55	5.94	-
Algal bioreactors (n = 3)	4.90 ± 0.22^{b}	5.42 ± 0.31^{a}	-	4.90 ± 0.43^{a}	5.66 ± 0.77^{a}	+	4.18 ± 0.04^{b}	5.35 ± 0.04^{a}	+
Bacterial bioreactors (n = 3)	4.91 ± 0.45^{ab}	5.54 ± 0.70^{a}	-	5.03 ± 0.63^{a}	5.54 ± 0.67^{a}	+	4.25 ± 0.11^{b}	5.21 ± 0.03^{a}	+
Algal clarifier	4.74	4.81	-	4.91	5.96	+	4.14	5.07	+
Bacterial clarifier	4.72	6.08	-	5.91	6.74	+	4.31	5.17	+
Algal filter	4.29	5.00	-	4.85	5.87	+	3.56	5.12	+
Bacterial filter	3.93	5.13	-	5.83	6.64	+	4.09	5.16	+
Algal UV	0.93	3.61	-	2.83	4.17	+	3.42	4.25	-
Bacterial UV	1.57	3.05	-	2.11	4.25	+	2.51	4.12	-
Holding tank (Algal)	3.24	5.62	-	3.72	5.60	+	2.94	5.69	+
Holding tank (Bacterial)	2.27	5.03	-	4.87	5.44	+	2.85	5.20	-
Holding tank (Control)	0.00	3.26	-	2.30	3.40	-	2.14	3.69	-

scale: 7, 6, 5, 4, 3, 2, 1, 0

Figure 4.6: Enumeration of bacteria and Salmonella detection in the treatment system before, during, and after 10^5 CFU mL $^{-1}$ Salmonella inoculation. Values in bioreactors are the means ± SD of three biological replicates (n = 3). Within each phase, means with similar letters are not statistically different. + and – represent presence and non-detection of Salmonella respectively.

As the dosing continued, however, *Salmonella* counts increased gradually to approximately 4.5 $\log_{10}$ CFU mL $^{-1}$ (~68.4% reduction) in all water samples from the bioreactors, clarifiers, and filters and this is consistent with the predictions of the mass balance model (> 4.7 $\log_{10}$ CFU mL $^{-1}$; Figure **4.5**B). This suggests that the bioreactor systems which were effective at 10^3 CFU mL $^{-1}$ dosing, began to lose their effectiveness in *Salmonella* removal as the high rate of dosing continued over a 10-day timeframe. Whereas before, *Salmonella* were <1% of all heterotrophs in the bioreactors as measured by APC, now they were in the majority, potentially blunting the opportunity for out-competition from other heterotrophs. The diminished treatment efficacy observed during prolonged high *Salmonella* dosing suggests a fundamental restructuring of the

microbial community in the wastewater treatment system and the ultimate effect was a decline in *Salmonella* removal.

Throughout the inoculation phase, there was no viable count of *Salmonella* in water samples downstream of UV disinfection, mirroring observations from the 10^3 CFU mL $^{-1}$ dosing experiment (Figure **4.3**). However, enrichment of water samples from post-inoculation (day 21) confirmed the presence of *Salmonella* at all sampling points up to but not including the hydroponic grow bed (Figure **4.6**). That *Salmonella* made it past the UV unit suggests either imperfect inactivation or bacteria reactivation, repair, and reproduction after light-based disinfection [157–160]. Gayán et al. [161] showed that photoreactivation of *Salmonella* is possible wherein exposure of *Salmonella* to visible light (11.5 klux) after UV light led to higher plate counts than UV treatment alone. However, as the Poultryponics grow beds were covered, the water in these systems is generally well protected from visible light, lowering the risk of light-facilitated photoreactivation.

Additionally, high levels of ammonium and orthophosphate in the treated water may have facilitated *Salmonella* recovery from UV-induced damage. Shafaei et al. [160] reported that nutrient availability (ammonia = 0.03 – 0.11 mg L^{-1} and orthophosphate = 1.0 – 1.5 mg L^{-1}) facilitated the photoreactivation of total coliforms in municipal wastewater effluent. In this study, a much higher nutrient concentration was detected in the treated effluents in the water holding tanks upstream of the lettuce grow beds (NH_4–N = 52 – 60 mg L^{-1}, PO_4–P = 9 – 11 mg $L^{-1}$and SO_4–S = 55 – 90 mg L^{-1}; Table **4.5**) indicating that nutrient availability could have contributed to the repair of photo-damaged *Salmonella* DNA. Unfortunately, N and P nutrients are essential for plant production and removal of these nutrients is therefore not a viable containment strategy.

Table 4.5: Average nutrient concentrations of treated poultry processing wastewater in the temporary holding tanks after UV disinfection.

Parameters	10^3 log $_{10}$ CFU mL $^{-1}$		10^5 log $_{10}$ CFU mL $^{-1}$	
	Algal system	Bacterial system	Algal system	Bacterial system
NH_4-N (mg L^{-1})	59.9 ± 21.5	58.3 ± 18.0	52.7 ± 7.9	52.5 ± 9.0
PO_4-P (mg L^{-1})	10.9 ± 2.9	10.8 ± 2.5	10.2 ± 1.2	10.5 ± 1.4
SO_4-S (mg L^{-1})	87.6 ± 25.8	89.3 ± 19.7	62.5 ± 14.3	54.1 ± 8.5
K^+ (mg L^{-1})	52.4 ± 18.0	51.0 ± 15.7	41.4 ± 5.1	41.4 ± 4.5
Na^+ (mg L^{-1})	139.7 ± 42.3	137.2 ± 40.2	121.6 ± 10.7	141.4 ± 40.0
Cl^- (mg L^{-1})	70.0 ± 8.2	71.3 + 7.1	67.3 ± 8.9	69.0 ± 11.7

While UV disinfection rendered *Salmonella* viable but non-culturable, its presence in the non-aerated water holding tanks raises concerns over potential contamination of downstream lettuce. *Salmonella* can be internalized into lettuce plants through damaged roots [162,163] and open stomata [164,165]. However, there was no detection of *Salmonella* in any lettuce samples that were harvested and analyzed after the inoculation phase of the second experiment (harvest 2, Table **4.6**). Our whole-head sampling methodology should capture both surface-attached and internalized pathogens. Likewise, the water of the grow beds tested negative for *Salmonella*. Similar to the upstream bioreactors, die-off in this compartment may be linked to a highly oxic environment coupled with a lack of organic substrates [100]. Thus, sustained aeration and covering of grow beds are likely effective strategies for *Salmonella* suppression. Where that proves insufficient due to an extreme upstream event, a post-UV disinfection approach using low levels of chlorine or PAA may be warranted. That said, *Salmonella* has been shown to be more resistant to chlorine disinfection compared to other indicator bacteria, such as total coliforms and *Enterococcus* [166]. Li et al. demonstrated that *Salmonella* could regrow even after chlorination at a dose of 69 mg·min L^{-1}, particularly following 24-hour dark storage of reclaimed water [166].

Table 4.6: Bacterial enumeration and isolation of pathogenic bacteria from lettuce samples cultivated during 10^5 CFU mL $^{-1}$ *Salmonella* inoculation.

Harvest	Lettuce	$\log_{10}$ CFU/g			*Salmonella*	*Campylobacter*
		E. coli	Total coliforms	Aerobic plate count		
1 (Day 10)	Algal system (n=6)	0 ± 0 [a]	4.22 ± 0.51 [a]	5.05 ± 0.52 [a]	nd	nd
	Bacterial system, (n=6)	0 ± 0 [a]	4.17 ± 0.23 [a]	4.81 ± 0.26 [a]	nd	nd
	Control (n=6)	0 ± 0 [a]	4.21 ± 0.41 [a]	4.86 ± 0.22 [a]	nd	nd
2 (Day 21)	Algal system (n=6)	0 ± 0 [a]	4.50 ± 0.42 [a]	5.37 ± 0.32 [a]	nd	nd
	Bacterial system, (n=6)	0 ± 0 [a]	3.87 ± 0.27 [a]	5.03 ± 0.31 [a]	nd	nd
	Control (n=6)	0 ± 0 [a]	3.99 ± 0.62 [a]	5.28 ± 0.44 [a]	nd	nd
3 (Day 31)	Algal system (n=6)	0 ± 0 [a]	3.10 ± 0.52 [b]	4.63 ± 0.36 [a]	nd	nd
	Bacterial system, (n=6)	0 ± 0 [a]	3.77 ± 0.46 [ab]	4.60 ± 0.19 [a]	nd	nd
	Control (n=6)	0 ± 0 [a]	4.17 ± 0.49 [a]	4.75 ± 0.17 [a]	nd	nd

Values are the means ± SD of three biological replicates with two samples per replicate (n = 6). Within each harvest, means with similar letters are not statistically different based on the Tukey's test ($p>0.05$).
nd : not detected after enrichment

At the end of the recovery phase (day 31), aerobic plate counts and total coliforms in each treatment unit had been restored to baseline levels, similar to those of the pre-inoculation phase. Nonetheless, enrichment assays on water samples indicated the persistence of *Salmonella* in all bioreactors, clarifiers, and filters (Figure **4.6**). Although *Salmonella* was no longer detected in the effluents from UV disinfection, algal-treated water samples in the holding tank downstream of UV disinfection still tested positive after the recovery phase. Slide agglutination test revealed that the isolate belonged to *S.* Typhimurium (O:1,4,5,12; Table **4.7**). Gayán et al. [161] reported that UV resistance varied amongst different strains of *Salmonella*. It was reported that the UV dose required to inactivate 99.99% of *S.* Typhimurium STCC 878 at ~10^8 CFU mL $^{-1}$ was 18.03 J mL $^{-1}$. It is noteworthy that *S.* Typhimurium O:1,4,5,12 exhibited similar resistance even at a higher UV dose in our treatment system (~24 J mL^{-1}). This suggests that certain *Salmonella* strains such as *S.* Typhimurium O:1,4,5,12, may exhibit increased resistance to UV treatment as observed in this study. These findings highlight the need for further research into strain-specific differences in UV susceptibility to enhance disinfection strategies in bioponic systems.

Table 4.7: Plate agglutination test of *Salmonella* isolates detected in water sample downstream of UV disinfection at the end of recovery phase following 10^5 CFU mL $^{-1}$ *Salmonella* inoculation.

Enrichment broth with presumptive colonies on XLT4	Antiserum	
	Poly A – I&Vi	O:4,5,27
BPW	+	+
RV	+	+
TT	+	+

BPW: Buffered peptone water
RV: Rappaport Vassiliadis broth
TT: Tetrathionate broth
+: positive

Because the water holding tank tested positive, water samples from the hydroponic grow beds were immediately collected and analyzed the same day for possible contamination. The analyses revealed no detection of *Salmonella*, *E. coli* and *Campylobacter* in the hydroponic reservoirs (Table **4.8**). Instead, approximately 4 $\log_{10}$ CFU mL $^{-1}$ of aerobic plate counts were detected in the water of the grow beds, confirming the presence of an active aerobic microbial community. This is consistent with our previous research on the Poultryponics system which showed robust nitrification was occurring in the plant grow bed [100]. Nitrification is essential for converting toxic ammonium into more suitable forms of nitrogen, primarily nitrate, crucial for crop production [21,25,65]. Thus, it is not desirable to eliminate all microorganisms in the plant grow bed (Figure **4.5**). It's also noteworthy that the plant grow beds were continuously aerated (dissolved oxygen concentration = ~21% air saturation, Figure **4.7**), suggesting that the highly oxygenated conditions could have played a role in inhibiting pathogen proliferation. Moreover, the treated poultry processing wastewater reaching the grow beds was devoid of soluble chemical oxygen demand (sCOD), eliminating a potential carbon source for pathogen survival [160]. Our earlier report highlighted that high heterotrophic activity within the upstream bioreactors was instrumental in achieving consistent sCOD removal rates (>80%) during the long-term operation of the Poultryponics system [100].

Similarly, *E. coli*, *Campylobacter,* and *Salmonella* were not detected in any of the lettuce samples harvested after the recovery phase (harvest 3, Table **4.6**). Notably, the counts for aerobic bacteria (4.6 – 4.8 $\log_{10}$ CFU g^{-1}) and total coliforms (3.1 – 4.2 $\log_{10}$ CFU g^{-1}) were comparable to those observed in earlier harvests. This consistency underscores the robustness of the Poultryponics treatment system in mitigating pathogen presence in lettuce, despite extreme dosing of *Salmonella* into the front end of the treatment system.

Table 4.8: Enumeration and isolation of pathogenic bacteria in hydroponic reservoirs at the end of the recovery phase following 10^5 CFU mL $^{-1}$ Salmonella inoculation.

Hydroponic grow bed	$\log_{10}$ CFU mL $^{-1}$			*Salmonella*	*Campylobacter*
	E. coli	Total Coliforms	Aerobic Plate Count		
Algal (n = 3)	0 ± 0 [a]	2.15 ± 0.13 [a]	3.35 ± 0.30 [b]	nd	nd
Bacterial (n = 3)	0 ± 0 [a]	2.20 ± 0.13 [a]	4.12 ± 0.14 [a]	nd	nd
Control (n = 3)	0 ± 0 [a]	1.72 ± 0.10 [b]	3.76 ± 0.20 [ab]	nd	nd

Values in bioreactors are the means ± SD of three biological replicates (n = 3). Means with similar letters are not statistically different based on the Tukey's test ($p>0.05$); nd: not detected after enrichment

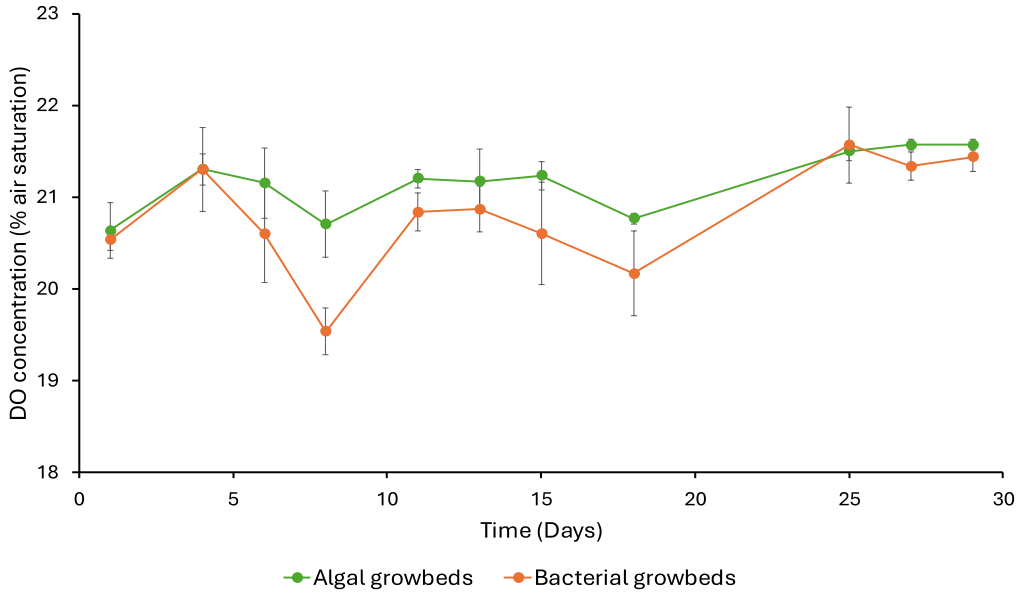


Figure 4.7: Dissolved oxygen concentration (% air saturation) in hydroponic grow beds fed with water from the algal treatment train (green) and bacterial treatment train (orange).

Another notable aspect of this pathogen-dosing study is the cultivation of hydroponic lettuce free of detectable *Salmonella* without resorting to advanced technologies such as ultrafiltration (UF) and reverse osmosis (RO). These technologies, while effective in reducing pathogen levels and other contaminants, often come with high operational and maintenance costs that are likely to be prohibitive for many agricultural operations. An economic evaluation of UF and RO for recycling poultry slaughterhouse wastewater in Texas, U.S. reported that UF and RO increased the annual wastewater treatment cost to ~ 8.6 million USD compared to ~ 0.82 million USD in conventional wastewater treatment [132]. The latter relies on biological treatment and simple solids separations (conventional activated sludge process), which is more similar to the Poultryponics system tested here [37,132].

Our findings demonstrate the capability of Poultryponics to repurpose wastewater for food production without compromising food safety. However, when the concentration of *Salmonella* approached that of other heterotrophs in the biological treatment systems, the effectiveness of the system began to diminish. Fortunately, such a scenario is highly improbable since bacterial populations in overflow chiller water are significantly reduced due to high dosage (< 2000 mg L^{-1} PAA) of antimicrobial chemicals [112]. This study expands the potential application of bioponics across various meat processing industries, where it has not previously been implemented, often due to concerns about pathogens [165].

However, prior to commercial implementation of bioponics that uses meat processing wastewater, further safety evaluations are required. These include evaluation of antibiotics, antimicrobial agents, and antibiotic resistance genes. Furthermore, although the high levels of *Salmonella* dosed in this study are highly improbable in any real poultry wastewater stream, the fact that our system broke down after sustained dosing at 5 Log CFU ml^{-1} *Salmonella* suggests a

need for risk assessment. If *Salmonella* do in fact reach the lettuce, they have the potential to internalize into its structure [140], making disinfection virtually impossible.

4.4 Conclusion

This study successfully demonstrated the efficacy of the Poultryponics system in managing high *Salmonella* influx in poultry processing wastewater. When challenged with a daily dose of 3 $\log_{10}$ CFU mL $^{-1}$ *Salmonella*, counts of *Salmonella* were sporadically detected in the treatment system, but with significantly lower counts in the algal bioreactors (0.6 $\log_{10}$ CFU mL^{-1}; 99.6% reduction) compared to the bacterial bioreactors (1.4 $\log_{10}$ CFU mL^{-1}; 97.5% reduction) ($p = 0.03$). At the higher *Salmonella* concentration (5 $\log_{10}$ CFU mL $^{-1}$), *Salmonella* counts in bioreactors, clarifiers, and filters were reduced to approximately 3 $\log_{10}$ CFU mL $^{-1}$ by day 15 of inoculation, representing a 99% reduction. As the high dosage continued, *Salmonella* removal in the bioreactors reduced to ~68.4% (~4.5 $\log_{10}$ CFU mL $^{-1}$) by day 20. Moreover, enrichment methods indicated potential strain-specific *Salmonella* persistence or reactivation after UV disinfection, however, no bacterial contamination (*Salmonella*, *E. coli*, and *Campylobacter*) was detected in grow bed water or any lettuce samples. The Poultryponics system also demonstrated robustness in restoring baseline pathogen levels after *Salmonella* inoculation ceased. These findings confirms that Poultryponics is effective at managing high *Salmonella* influxes without the need for advanced filtration technologies. Such findings may translate to treatment of wastewater from other agricultural and food production industries.

Chapter 5: Impact of system design and operation on microbial community structure affecting organic degradation, nitrogen transformation, plant growth promotion, and food safety in Poultryponics

Impact of system design and operation on microbial community structure affecting organic degradation, nitrogen transformation, plant growth promotion, and food safety in Poultryponics

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Highlights

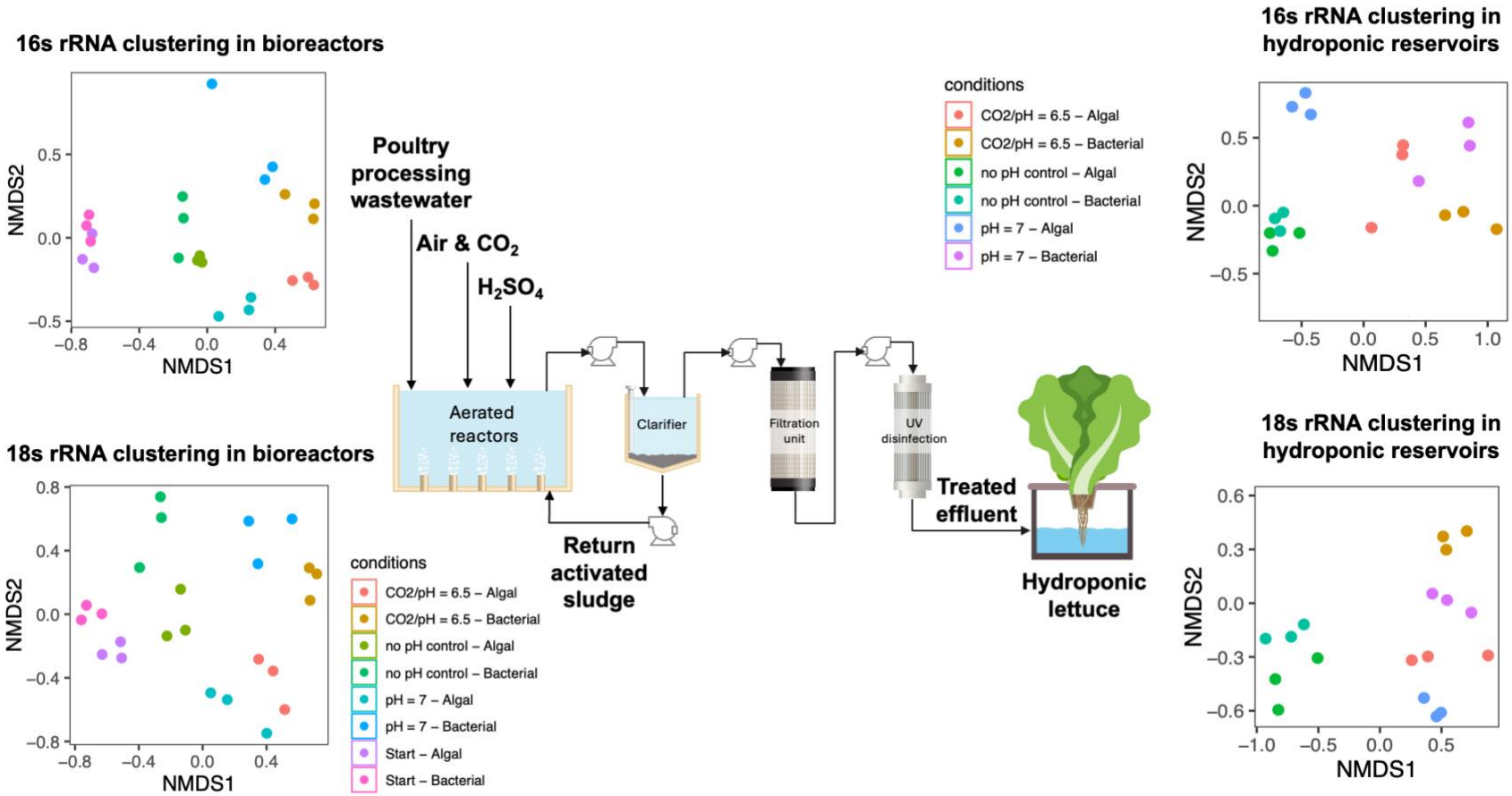
1. Bioreactor operational strategies influenced microbial diversity
2. Nitrifier abundance was limited in bioreactors but enhanced in hydroponic reservoirs
3. Acid dosing shifted nitrogen transformation from denitrification to DNRA
4. Siderophore activity and low levels of plant-promoting metabolites were detected in the hydroponic reservoirs

Abstract

Poultryponics is a system that integrates poultry processing wastewater (PPW) treatment with hydroponic crop production. This study investigated how system design, specifically bioreactor illumination, pH control and CO_2 supplementation, affected microbial community structure and function. Two parallel treatment trains were run for ~220 days: the first train utilized illuminated bioreactors that were supplied with CO_2 to enable algae growth while the second train used covered reactors to suppress algae growth. High organic content in PPW (~562 mg L^{-1} sCOD) favored heterotrophic growth in all bioreactors, with Proteobacteria (46.3–83.0% of prokaryotes), Bacteroidetes (6.5–20.6% of prokaryotes) and fungal decomposers facilitating organic matter degradation (>80% sCOD removal). Illumination and CO_2 sparging promoted *Desmodesmus* (~45.8% of eukaryotes) in the algal bioreactors. Nitrifier abundances were low in all bioreactors (<0.03%) but were increased in hydroponic reservoirs (<4.31%) due to pH-modulating effects of CO_2 and align with observed trends in nitrification. The hydroponic reservoirs exhibited siderophore activity, with significantly higher deferoxamine mesylate equivalence in the algal system (~0.6 ng L^{-1}, $p < 0.05$), but this did not significantly increase iron content in plants. These findings demonstrate how system operation influences synergistic interactions within microbial communities, enhancing nutrient cycling, plant growth promotion and food safety.

Keywords: Bioponics; Microbial community analysis; Nitrification; Nutrient cycling; Plant growth-promotion; Wastewater treatment

Graphical abstract



5.1 Introduction

Poultryponics is a sustainable technology that recycles nutrients (~80 mg N L^{-1}, ~20 mg P L^{-1}, and ~70 mg K L^{-1}) present in poultry processing wastewater (PPW) for hydroponic crop production [100]. However, PPW contains reduced forms of nitrogen (~100 mg L^{-1} total Kjeldahl nitrogen), volatile fatty acids (VFAs; ~1000 mg L^{-1}), proteins (~450 mg L^{-1}), and lipids (~40 mg L^{-1}) [10]. Therefore, treating PPW to reduce the organic load and transform the nitrogen into nitrate is essential prior to hydroponic irrigation to ensure stable crop production.

Poultryponics relies on microbial consortia to degrade these complex organic compounds in PPW while converting ammonium to nitrate to support crop production. During ammonification, nitrogenous organic compounds such as proteins and amino acids are mineralized into ammonium (NH_4^+) [76]. Subsequently, nitrification, a two-step aerobic process governed by ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), converts ammonium to nitrate (NO_3^-) [167]. Nitrate is the primary form of nitrogen essential for plant growth because plants often cannot utilize organic nitrogen and are intolerant of high ammonium-N levels [103,168].

Several studies have demonstrated that photoautotrophs, such as microalgae, can enhance nitrification by increasing dissolved oxygen (DO) via photosynthesis [169,170], supporting both heterotrophic and autotrophic communities in wastewater treatment systems [46]. For instance, the presence of *Auxenochlorella protothecoides* in PPW under batch conditions significantly increased nitrifying bacteria abundance, leading to a 4 – 9-fold increase in nitrate production [47]. Beyond oxygenation, microalgae can sequester and degrade toxic compounds in high strength wastewaters such as volatile fatty acids [44] and phenolic compounds [45], which are inhibitory to nitrifying populations [171].

However, in our previous pilot-scale Poultryponics study conducted continuously for >200 days, nitrification was limited in all aerated bioreactors (illuminated to promote algae vs. no illumination) due to a combination of several factors [100]. These included high organic content in PPW, short retention time, and competition from fast-growing heterotrophs with higher oxygen affinities compared to nitrifying bacteria, especially under low DO levels (<3.5 mg L^{-1}). These conditions suppressed nitrification in the bioreactors, possibly shifting nitrogen transformations toward alternative pathways such as denitrification and dissimilatory nitrate reduction to ammonium (DNRA). Denitrification is an anaerobic process where facultative heterotrophic bacteria reduce nitrate (NO_3^-) to gaseous nitrogen species (N_2 or N_2O), leading to nitrogen loss from the system [172]. In contrast, DNRA retains nitrogen within the system by reducing nitrate to ammonium [173]. The balance between these nitrogen pathways is critical in Poultryponics, as it influences nitrogen retention and/or loss, and overall nutrient availability for plant uptake.

Furthermore, salinity and nutrient imbalances are of particular relevance to Poultryponics because PPW has high Na^+ (~150 mg L^{-1}) and Cl^- (~100 mg L^{-1}) content and was previously found to be deficient in several key plant nutrients such as Ca, Mg, and K [174]. Microbial communities can promote nutrient solubilization, improve nutrient uptake, enhance plant biomass, and increase stress tolerance against salinity, nutrient imbalances, and pathogenic infections in hydroponic systems [175,176]. Specifically, plant growth-promoting bacteria (PGPB) produce phytohormones like indole-3-acetic acid (IAA and cytokinins, as well as siderophores that improve micronutrient availability, thereby supporting plant growth and resilience [177–179]. As an example, iron availability is of particular interest in Poultryponics because past research showed that lettuce tissues were not deficient in iron despite low background concentrations in the treated wastewater of <0.25 mg L^{-1} [174]. In contrast, hydroponic solutions typically contain >2 mg L^{-1} of iron [73]. These results raise

important research questions surrounding the role of microorganisms in facilitating nutrient transformation and uptake, plant production, and food safety.

Several bioponic studies have explored the impact of microbial communities on organic degradation, nitrification, nutrient solubilization, and plant growth-promotion [65,180]. However, the specific impacts of the operational strategies in Poultryponics on microbial community composition and function (organic removal, nitrogen transformation, pathogen removal etc.) remain unclear. Therefore, the objectives of this study were to: (a) determine the impact of bioreactor conditions (illumination, CO_2 supplementation, and pH control) on the dominant eukaryotic and prokaryotic populations in Poultryponics; (b) assess the dynamics of nitrifying populations in bioreactors and hydroponic reservoirs and their association with the observed nitrification performance; (c) evaluate changes in other nitrogen cycle-related taxa and their potential impacts on nitrogen dynamics (loss/retention) within the system; (d) determine the impacts of operational strategies on plant growth-promotion and metabolites, impacting plant health and productivity; and (e) assess the abundance of microbial populations that have previously been implicated in food safety and quality issues in produce. It was hypothesized that operation strategies would restructures the microbial communities not only in bioreactors but also in the downstream hydroponic system. This study provides insights into how operational strategies affect microbial community structure, influence nitrogen transformations, and promote plant health in sustainable, wastewater-based hydroponic systems.

5.2 Materials and methods

5.2.1 Experimental setup and operation of Poultryponics

Poultryponics is a pilot-scale biological wastewater treatment system (~115 L d^{-1} capacity) coupled to an indoor hydroponic setup for simultaneous lettuce cultivation. The wastewater treatment system consists of two parallel trains: an algal-bacterial system and a bacteria-only system. Wastewater from a dissolved air flotation (DAF) unit at a commercial broiler processing facility was collected and pumped continuously into the treatment trains. Each treatment train comprised bioreactors (3 replicates; with illumination in the algal system and no illumination in the bacterial system), clarifiers, bag filters, UV disinfection, water holding tanks, and a deep-water system for lettuce cultivation (Figure **5.1**). All bioreactors were continuously aerated (100 ml min^{-1}), with the algal-bacterial bioreactors being supplied with CO_2 (0.5% v/v in air) to spur algal growth. In contrast, the bacterial bioreactors were covered with a dark cloth to suppress photosynthesis and algal growth. Treated effluent from each train supplied three aerated hydroponic reservoirs, each supporting six butterhead lettuce plants (18 plants per treatment). Two additional hydroponic treatments (3 replicates each) were included to evaluate potential nutrient deficiencies in Poultryponics: one received treated effluent from the algal system supplemented with mineral fertilizer, while the other served as a control using a nutrient solution representative of commercial hydroponic systems (Figure **2.1**). All hydroponic reservoirs were maintained under controlled lighting (640 mmol photons m^{-2} s^{-1} on an 8h:16h light-dark cycle) and temperature conditions (20 – 25°C). Detailed descriptions of all the system components and specifications have been published previously [100].

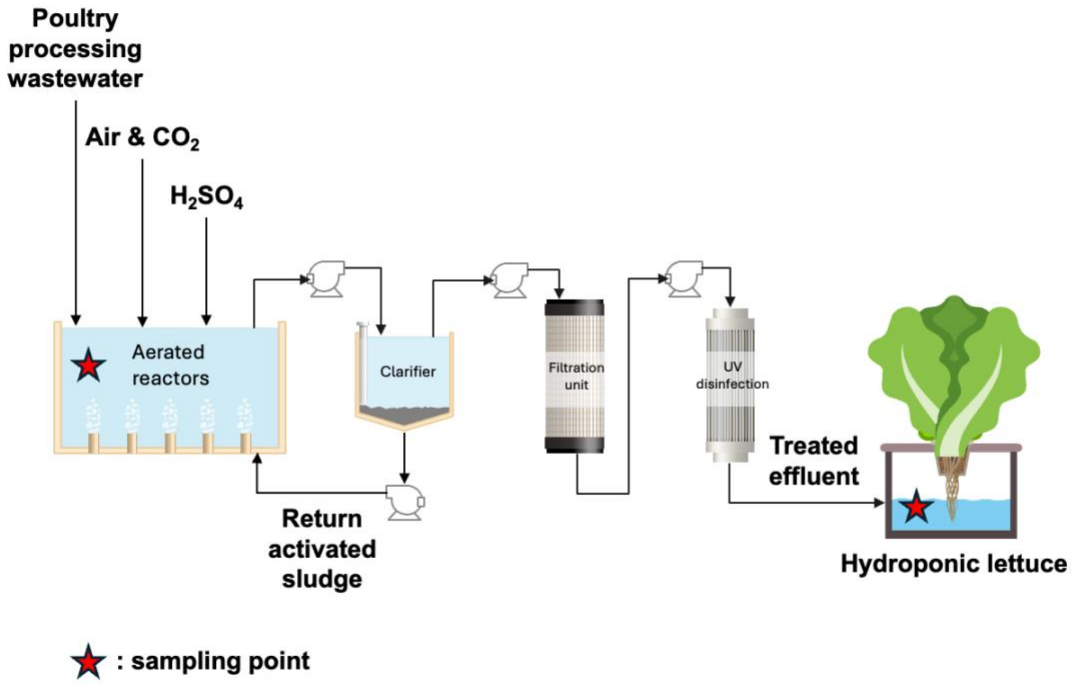


Figure 5.1: Schematic representation of Poultryponics showing water flow, lettuce production and sampling points. Microbial biomass was collected from the bioreactors and hydroponic reservoirs at the end of each operational condition: baseline operation (Phase I), upstream pH management with H_2SO_4 (Phase II) and CO_2 sparging in all bioreactors (Phase III).

The Poultryponics system was operated continuously from Fall 2022 to Summer 2023, spanning 222 days. The operation was divided into three phases to evaluate system performance under different conditions (Table **5.1**). In Phase I (Days 0 – 65), the system was operated with the baseline design to assess its performance in nutrient recovery and transformation. However, robust ammonification and organic matter degradation upstream of the hydroponic reservoirs resulted in elevated pH levels (7.5 – 8), exceeding the optimal range for hydroponic lettuce production (~6.0). In Phase II (Days 65 – 163), pH controllers were installed to dose 0.25 M H_2SO_4 into the bioreactors to maintain a set pH of 7. In Phase III (Days 163 – 222), CO_2 was sparged into all bioreactors to investigate the effects of alkalinity and inorganic carbon availability on nitrification.

5.2.2 Analytical methods and nitrogen balance

Water samples were collected from each treatment unit three times per week, filtered (0.22μm) and stored at -20°C. Water quality analyses were conducted using suppressed ion chromatography for soluble nitrogen species (NH_4-N, NO_2-N, and NO_3-N) and HACH assays for total nitrogen (TN) and soluble chemical oxygen demand (sCOD) as previously described [74]. Elemental analysis of water samples was performed by microwave digestion (CEM Mars 6 Microwave, Matthews, NC, USA) followed by inductively coupled plasma – optical emission spectrometry (ICP-OES) (Agilent 5800, Santa Clara, CA). Nitrification rates (mg N L^{-1} d^{-1}) was determined by NO_3-N accumulation (mg N L^{-1}) normalized by the sampling interval (days). Nitrogen loss (% of influent TN) was determined as the difference between the cumulative nitrogen inflow and outflow in the system, representing the total nitrogen lost from the system either by denitrification or volatilization [174].

Table 5.1: Summary of experimental conditions across operational phases in Poultryponics

Phase	Days	Objective	Conditions			
			pH set point	Illumination	CO_2	Lettuce
I	0 – 65	Baseline system performance	N/A	algal bioreactors only	algal bioreactors only	yes
II	65 – 163	pH control (acid dosing)	7.0	algal bioreactors only	algal bioreactors only	yes
III	163 – 222	CO_2 sparging for alkalinity and inorganic carbon adjustment	6.5	algal bioreactors only	all bioreactors	yes

5.2.3 16S and 18S ribosomal deoxyribonucleic acid (rDNA) amplicon sequencing

At the end of each operational phase (Phase I, II and III), the bioreactors and hydroponic reservoirs were thoroughly mixed and ~100mL of culture volume was collected. The sample was centrifuged at 4696 x g for 10 mins and resuspended in dH_2O three times to remove salts. The pellet was freeze-dried to determine total suspended solids (TSS) and stored at -80°C for DNA extraction as previously described [181]. DNA extraction of freeze-dried biomass pellets was conducted using the FastDNA Spin Kit (MP Biomedicals) according to the manufacturer's instructions. The QuantiFlour dsDNA system (Promega) was used for quantifying the extracted DNA concentration. Targeted amplicon of the 16S rRNA gene (primers: 515F and 806R) and 18S rRNA gene (primers: 1391F and EukBr) was performed by MR DNA (Molecular Research LP, Texas) using an Illumina MiSeq instrument. Polymerase Chain Reaction (PCR) was performed for 30 cycles using the HotStarTaq Plus Mix Kit (Qiagen, USA) under the following conditions: 95°C for 5 minutes, followed by 30-35 cycles of 95°C for 30 seconds, 53°C for 40 seconds, and 72°C for 1 minute. A final elongation step at 72°C for 10 minutes was performed. PCR products were checked using a 2% agarose gel, and the sequence data was processed using the sequencing center's ribosomal and functional gene analysis pipeline as previously described [74]. The original FASTQ files from this study have been uploaded to NCBI's Sequence Read Archive (SRA) under the accessions SUB15187513 and SUB15200387 for 16S and 18S rRNA sequences respectively.

5.2.4 Analysis for siderophore activity

Water samples collected from bioreactors and hydroponic reservoirs were centrifuged at 4696 x g for 10 mins, filtered (0.22μm) and stored at -80°C. Siderophore quantification was determined by mixing the water samples with Chrome Azurol S (CAS) at a 1:1 ratio and measuring

the absorbance in microplate reader at 630 nm [182]. A positive control with 15 µM deferoxamine mesylate (Desferal, Sigma) was used as a reference standard and for standard curve generation to quantify siderophore activity in the samples. Equation 5 was used to estimate siderophore production in percent siderophore unit (psu).

$$\text{Percent siderophore unit (psu)} = \frac{A_r - A_s}{A_r} \times 100 \qquad \text{Equation 5}$$

where: A_r and A_s are the abundance of the reference and sample respectively.

5.2.5 Cytokinin bioassays

Cytokinin activity was assessed in water-extracts from freeze-dried biomass samples in hydroponic reservoirs collected at the end of each operational phase (Phase I, II and III) using two complementary *Arabidopsis thaliana* bioassays: (i) GFP fluorescence in TCSn:GFP reporter lines [183], and (ii) a dark-induced leaf senescence assay based on photosystem II (PSII) efficiency (Fv/Fm) measurements [184]. Distilled water (DW) and 1 µM *trans*-zeatin (tZ) were used as negative and positive controls, respectively.

Seeds of TCSn:GFP *Arabidopsis thaliana* lines were surface sterilized and transferred to 4 °C for 3 days for stratification to synchronize germination. Seeds were plated on agar plates containing MS media (1x) + 1% sucrose. Plates were kept in 22 °C/ 18 °C, 16 h/8 h light (100µmol m^{-2} s^{-1})/dark cycle conditions. 15 days after germination, whole Arabidopsis seedlings were transferred to water-extracts from freeze-dried biomass samples collected from hydroponic reservoirs or DW as control. *trans*-Zeatin (tZ; 1 µM) was used as positive control. 24h after treatment, whole seedlings were transferred to a glass slide and fluorescing root tips visualized

using a Nikon Eclipse 80i microscope epifluorescence microscope with a UV source and a green fluorescent protein (GFP) filter. Using a Qimaging Fast 1394 digital camera root tip images were captures and presented without altering the original photo integrity.

Seeds of *Arabidopsis thaliana* (Col-0) were plated and grown similar to TCSn:GFP experiment. 15 days after germination, leaf 1 and 2 were excised and transferred to 6-well plate containing treatment water or DW as control. trans-Zeatin (tZ; 1 µM) was used as positive control. Leaves were floated on water-extracts from freeze-dried biomass samples collected from hydroponic reservoirs with adaxial side facing up and the plate covered with aluminum foil to maintain dark conditions. Plates were kept in 22 ℃/ 18 ℃, 16 h/8 h cycle conditions. After 20mins of dark-adaptation, Fv/Fm (a non-destructive photosystem II efficiency parameter) was recorded using an Open FluorCam (Photon Systems Instruments, Czech Republic). Similarly, Fv/Fm was recorded at 48h, 96h, and 144h after treatment.

5.2.6 LC-MS/MS analysis of plant growth-promoting compounds

LC-MS/MS analysis of water samples in the hydroponic reservoirs was performed at the Auburn University Mass Spectrometry Laboratory. The LC-MS/MS was a Vanquish UHPLC (Thermo Fisher, USA) coupled with an orbitrap mass spectrometer (Orbitrap Exploris 120, Thermo) using electrospray ionization (H-ESI) in both positive and negatives modes with Xcalibur software for data acquisition. A 1-µL injection of sample was made on a C18 column (ACQUITY UPLC® BEH C18, 1.7 µm, 2.1 × 50 mm, Waters) at 40 °C with a 200 µL/min flow rate of mobile phase solution A (99.9% water and 0.1% formic acid) and solution B (99.9% methanol and 0.1% formic acid). The MS scan range was set at 70-700 m/z with resolution of 120,000.

Untargeted analysis was performed to identify potential plant-growth modulating compounds. The compound selection criterion followed a requirement that they achieved an mzCloud annotation with a match factor >90%. Compounds meeting this criterion were further screened by performing a literature search for molecules associated with plant-growth modulation, including auxin signaling, stress response, and root architecture modification. Compounds that met these criteria were spiked into the original samples at 1 mg/L to confirm retention time and spectral match and an external calibration curve in methanol at 500 µg/L, 100 µg/L, 10 µg/L, 1 µg/L, and 0.1 µg/L were prepared. Spiked samples and standards were analyzed under the same LC-MS/MS conditions to confirm the presence based on retention time (±0.05 min) and spectral match. The external calibration curve was used for quantification.

5.2.7 Statistical Analyses

Microbial community analyses were conducted separately for prokaryotic (16S rDNA) and eukaryotic (18S rDNA) datasets to evaluate taxonomic clustering and compositional shifts across experimental phases using R statistical software v4.4.0. Alpha diversity indices (Shannon, Simpson, and Richness) were calculated from raw sequence counts using the 'vegan' package [185]. Non-metric multidimensional scaling (NMDS) was performed to analyze shifts in the community using the 'phyloseq' package. Similarity percentage breakdown analysis (SIMPER) was used to assess the differences in community structure between two selected operational phases using Bray-Curtis dissimilarity. Averages, standard deviations, standard errors and independent t-tests were carried out in Microsoft Excel. ANOVA and Tukey's HSD test were carried out in R using the 'agricolae' and 'car' packages. Data that violated homogeneity of variance were log transformed before Tukey's multiple comparison test.

5.3 Results and discussion

At the start of the experiment, all bioreactors were inoculated with a consortium from a biofloc aquaculture facility. Alpha diversity metrics (genus level) included: richness = 336 for prokaryotes and 103 for eukaryotes; Shannon index = 3.6 for prokaryotes and 2.0 for eukaryotes; and Simpson index = 0.9 for prokaryotes and 0.7 for eukaryotes. More detailed diversity metrics for prokaryotes and eukaryotes can be found in Table **5**.**2** and Table **5**.**3**.

As the experiments progressed, the microbial communities underwent significant restructuring due to the operational conditions in the bioreactors and their associated hydroponic reservoirs, which influenced community composition and their associated functions (Figure **5**.**2**). In the following sections, we connect microbial taxa to core functions observed within the Poultryponics system such as organic compound mineralization, photosynthesis in algal system, nitrogen transformation, plant growth-promoting secretions, and food safety.

5.3.1 Organic compound mineralization predominant in bioreactors

Due to the composition of PPW (high organic loading = 562 ± 167 mg L^{-1} sCOD; VFA = 313 ± 116 mg L^{-1}; TN = 59 ± 27 mg N L^{-1}; Appendix, Table A4), there was high heterotrophic activity in all bioreactors across all 3 phases. This was characterized by high sCOD removal (>82% for all bioreactor types across all 3 phases) and robust ammonification and N retention (NH_4-N = 58 – 72 mg N L^{-1}, Table **5**.**4**).

Proteobacteria were the dominant phylum throughout the study, accounting for 46.3 – 83.0% of the microbial community in both bioreactor systems (Figure **5**.**3**A). Several genera of Proteobacteria including *Brachymonas* (17.7%), *rhodocyclus* (9.0%) and *desulfomicrobium* (4.9%) were present in the bioreactors (Appendix, Table A5).

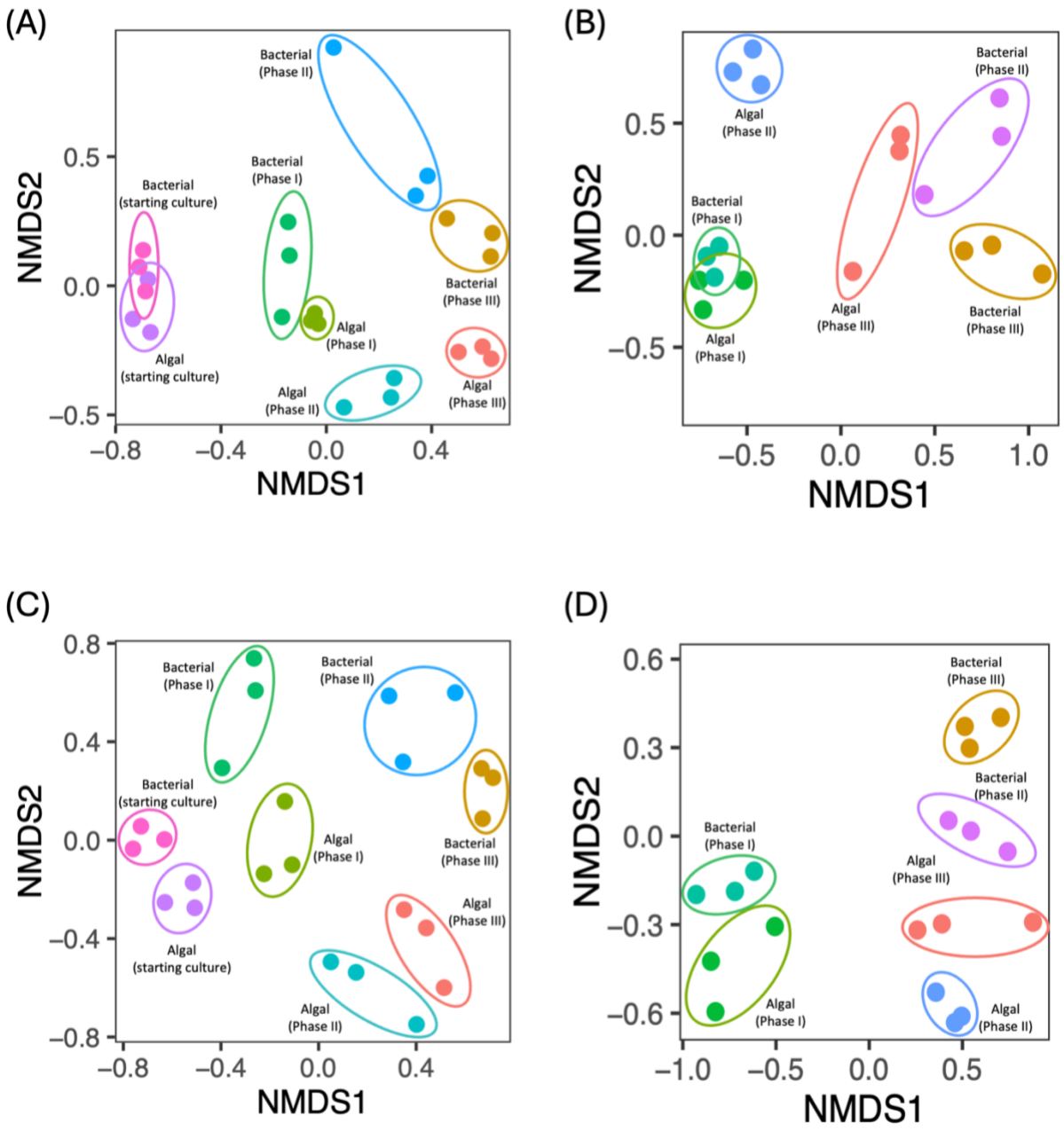


Figure 5.2: Non-metric multi-dimensional scaling (NMBS) plots of genus-level microbial community clustering based on 16S rRNA and 18S rRNA gene profiles in bioreactors (A & C) and hydroponic reservoirs (B & D) across operational phases.

Table 5.2: Alpha diversity indices at the genus level for prokaryote communities in Poultryponics across operational phases.

	Alpha diversity indices		
	Richness	Shannon	Simpson
Bioreactors			
Algal: starting culture	338.3 ± 25.7[b]	3.6 ± 0.3[b]	0.923 ± 0.019[b]
Algal: Phase I	459.7 ± 8.0[a]	4.5 ± 0.2[a]	0.972 ± 0.015[a]
Algal: Phase II	431.7 ± 25.4[a]	4.4 ± 0.3[a]	0.966 ± 0.019[a]
Algal: Phase III	454.0 ± 23.1[a]	4.4 ± 0.3[a]	0.963 ± 0.014[a]
Bacterial: starting culture	335.0 ± 18.5[b]	3.6 ± 0.4[a]	0.914 ± 0.032[a]
Bacterial: Phase I	391.7 ± 26.0[ab]	4.0 ± 0.8[a]	0.908 ± 0.118[a]
Bacterial: Phase II	354.0 ± 68.1[ab]	3.8 ± 0.5[a]	0.942 ± 0.030[a]
Bacterial: Phase III	416.0 ± 15.9[a]	4.2 ± 0.2[a]	0.967 ± 0.011[a]
Hydroponic reservoirs			
Algal: Phase I	298.7 ± 9.1[a]	3.8 ± 0.2[a]	0.933 ± 0.023[a]
Algal: Phase II	307.0 ± 14.0[a]	3.8 ± 0.6[a]	0.921 ± 0.048[a]
Algal: Phase III	296.3 ± 44.8[a]	3.8 ± 0.5[a]	0.934 ± 0.049[a]
Bacterial: Phase I	312.3 ± 8.1[a]	4.2 ± 0.3[a]	0.955 ± 0.025[a]
Bacterial: Phase II	225.3 ± 32.7[b]	3.7 ± 0.1[b]	0.945 ± 0.003[a]
Bacterial: Phase III	237.0 ± 15.4[b]	3.7 ± 0.3[b]	0.946 ± 0.014[a]

Values are means ± standard deviation of 3 biological replicates. Within each treatment type (algal or bacterial), means with similar letters are not statistically different based on the Tukey's HSD test ($p>0.05$).

The metabolic versatility and role of Proteobacteria in organic matter degradation (VFA utilization) and nutrient cycling are well-documented in soil and bioponic systems [21,186]. Similar studies have shown Proteobacteria's prominence in biofilters, freshwater aquaculture, and wastewater treatment systems due to their role in nitrogen and phosphorus cycling [187,188]. Bacteroidetes, the second most dominant phylum (6.5 – 20.6%) in the bioreactors, further supports

hydrolysis and degradation of complex organic compounds [188]. These two phyla likely complemented each other in breaking down complex organic substrates such as urea, free amino acids, and nucleic acids present in PPW into bioavailable forms (NH_4-N), facilitating high sCOD removal (>80%) in Poultryponics [100].

Table 5.3: Alpha diversity indices at the genus level for eukaryote communities in Poultryponics across operational phases.

	Alpha diversity indices		
	Richness	Shannon	Simpson
Bioreactors			
Algal: starting culture	99.7 ± 15.5^{a}	2.0 ± 0.3^{a}	0.773 ± 0.065^{a}
Algal: Phase I	89.7 ± 4.9^{a}	1.9 ± 0.2^{a}	0.725 ± 0.011^{a}
Algal: Phase II	85.0 ± 3.0^{a}	2.1 ± 0.2^{a}	0.807 ± 0.040^{a}
Algal: Phase III	80.0 ± 10.4^{a}	1.3 ± 0.5^{a}	0.489 ± 0.156^{b}
Bacterial: starting culture	106.0 ± 9.2^{a}	1.9 ± 0.6^{ab}	0.646 ± 0.207^{ab}
Bacterial: Phase I	99.0 ± 10.6^{b}	2.2 ± 0.4^{a}	0.793 ± 0.072^{a}
Bacterial: Phase II	62.3 ± 14.0^{c}	1.2 ± 0.4^{b}	0.454 ± 0.193^{b}
Bacterial: Phase III	96.7 ± 14.4^{b}	2.1 ± 0.3^{a}	0.727 ± 0.072^{a}
Hydroponic reservoirs			
Algal: Phase I	60.0 ± 10.8^{b}	1.3 ± 0.2^{b}	0.608 ± 0.090^{b}
Algal: Phase II	101.3 ± 7.0^{a}	2.6 ± 0.1^{a}	0.851 ± 0.022^{a}
Algal: Phase III	109.3 ± 31.5^{a}	2.5 ± 1.1^{a}	0.797 ± 0.221^{ab}
Bacterial: Phase I	73.7 ± 18.8^{a}	1.2 ± 0.2^{b}	0.524 ± 0.124^{b}
Bacterial: Phase II	83.0 ± 10.8^{a}	2.4 ± 0.4^{a}	0.826 ± 0.076^{b}
Bacterial: Phase III	96.3 ± 10.3^{a}	2.2 ± 0.4^{a}	0.768 ± 0.124^{b}

Values are means ± standard deviation of 3 biological replicates. Within each treatment type (algal or bacterial), means with similar letters are not statistically different based on the Tukey's HSD test ($p>0.05$).

Table 5.4: Summary of treatment performance of Poultryponics during 222 days of operation

Parameters	Algal-bacterial bioreactors			Bacterial bioreactors		
	Phase I	Phase II	Phase III	Phase I	Phase II	Phase III
NH_4-N (mg L^{-1})	71.6 ± 28.4[a]	58.0 ± 10.9[a]	71.3 ± 28.8[a]	60.2 ± 22.2[a]	64.4 ± 12.3[a]	68.4 ± 19.8[a]
NO_2-N (mg L^{-1})	0.5 ± 0.4[a]	0.1 ± 0.1[a]	0.1 ± 0.1[a]	1.9 ± 1.2[a]	0.1 ± 0.1[a]	0.0 ± 0.0[a]
NO_3-N (mg L^{-1})	1.5 ± 0.8[a]	0.3 ± 0.1[a]	0.1 ± 0.1[a]	2.1 ± 1.6[a]	0.1 ± 0.1[a]	0.1 ± 0.1[a]
sCOD removal (%)	90.3 ± 0.1[a]	88.8 ± 0.1[a]	84.4 ± 0.1[a]	91.8 ± 0.1[a]	88.6 ± 0.1[a]	82.9 ± 0.1[a]
Nitrification rate (mg N L^{-1} d^{-1})	0.1 ± 0.1[b]	0.1 ± 0.1[a]	0.1 ± 0.1[a]	0.5 ± 0.2[a]	0.1 ± 0.1[a]	0.1 ± 0.1[a]
Nitrogen loss (% influent TN) *	18.7	3.2	2.6	23.6	0.8	18.1
Parameters	Algal-bacterial plant reservoirs			Bacterial plant reservoirs		
	Phase I	Phase II	Phase III	Phase I	Phase II	Phase III
NH_4-N (mg L^{-1})	8.2 ± 4.2[a]	4.9 ± 2.4[b]	18.2 ± 9.1[b]	7.0 ± 4.6[a]	63.1 ± 15.1[a]	34.3 ± 16.4[a]
NO_2-N (mg L^{-1})	29.2 ± 20.4[a]	0.5 ± 0.3[a]	0.2 ± 0.2[a]	20.3 ± 17.0[a]	0.1 ± 0.1[b]	0.4 ± 0.3[a]
NO_3-N (mg L^{-1})	51.4 ± 25.9[a]	51.5 ± 16.6[a]	44.0 ± 16.0[a]	54.5 ± 28.2[a]	11.8 ± 3.8[b]	36.0 ± 18.9[a]
Nitrification rate (mg N L^{-1} d^{-1})	6.3 ± 4.4[a]	8.6 ± 6.3[a]	6.2 ± 4.2[a]	8.7 ± 5.8[a]	1.2 ± 0.8[b]	6.1 ± 2.6[a]
Nitrogen loss (% influent TN) *	8.1	9.8	2.8	12.7	2.8	5.5

Values are the means ± standard deviation of 3 biological replicates averaged over the duration for each operational condition.
Means with the same letters between the algal and bacterial systems within each operational phase, were not statistically significant based on Tukey HSD ($p>0.05$).
*: Statistical analysis was not performed on nitrogen losses because they were calculated based on the total N flow into the system via PPW and the cumulative N flow out (NH_4-N, NO_2-N, NO_3-N, and org-N) of the three biological units.

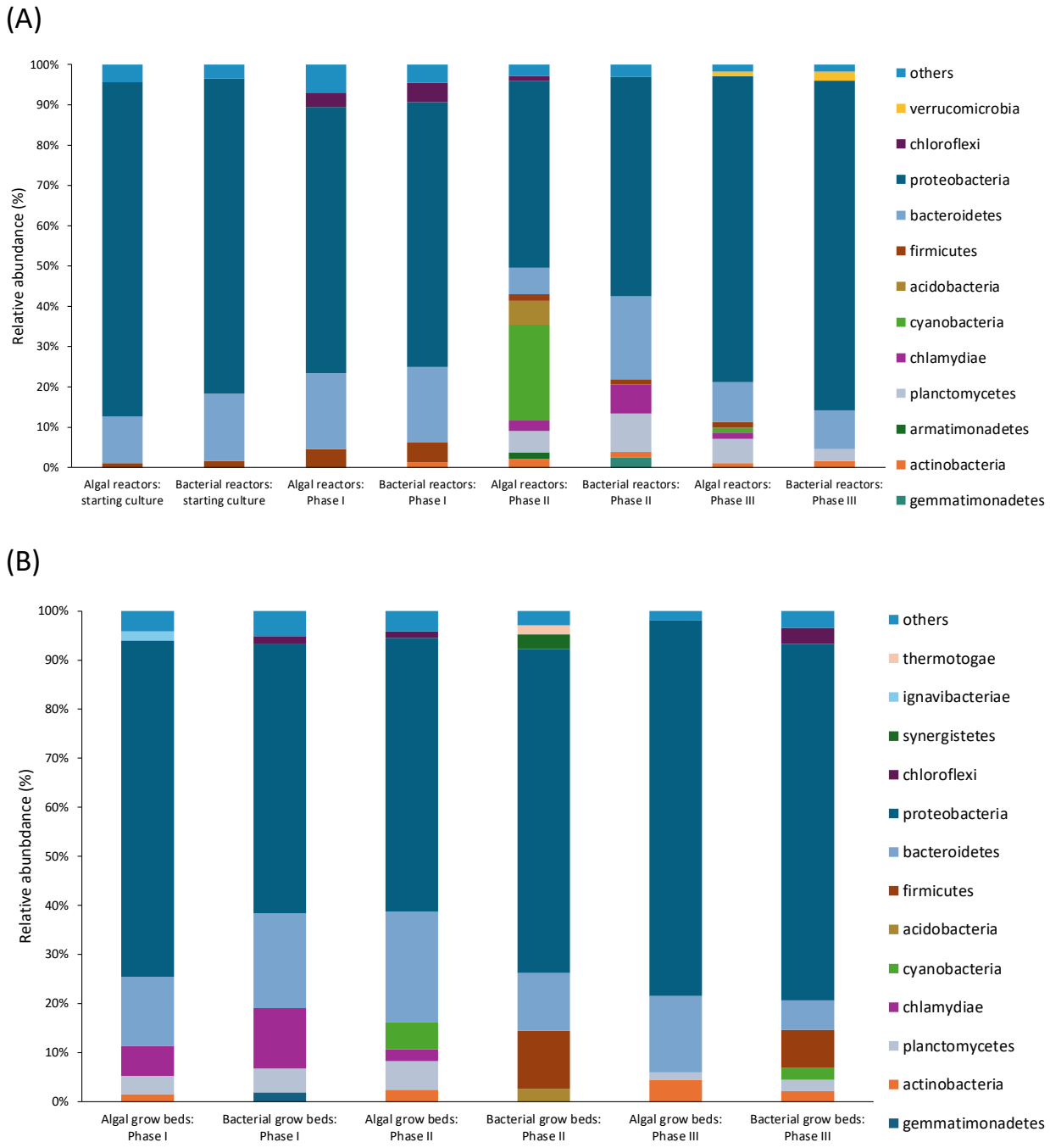


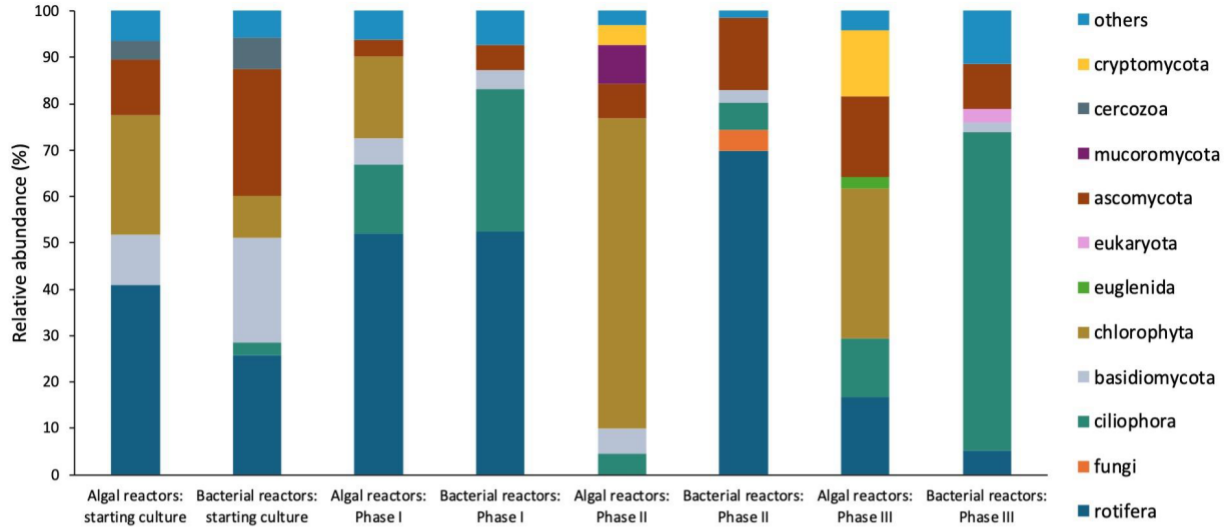
Figure 5.3: Relative 16S rRNA gene abundance at the phylum level in bioreactors (A) and hydroponic reservoirs (B) based on n=3 biological replicates. Phyla with relative abundance below 1% were assigned as others.

However, elevated pH levels (7.5 – 8) resulting from robust organic carbon degradation and ammonification contributed to low lettuce yields in the hydroponic reservoirs [174]. Therefore, pH controllers were installed to dose 0.25M H_2SO_4 into the bioreactors to maintain a pH of 7.0 in Phase II to optimize lettuce growth. This operational change resulted in a marginal reduction of sCOD removal in both bioreactor systems (~89%, Table **5.4**). Acid dosing significantly impacted microbial diversity in the bacterial bioreactors in Phase II, where richness, Shannon and Simpson indices of eukaryote communities significantly decreased (Table **5.3**). The predominant acid-sensitive taxa in the bacterial bioreactors were members of the phylum Firmicutes (5.0% in Phase I and 1.3% in Phase II, Figure **5.3**A); commonly found in chicken manure [65] and fecal/gut microbiota [189]. Declines in Proteobacteria following acid dosing were largely driven by a large decline in the genus *Brachymonas* (17.7% in Phase I and 1.3% in Phase II, Appendix, Table A6); a common denitrifying bacterial genus in wastewater treatment plants [190].

Meanwhile, fungal communities in bacterial reactors showed resilience under acidic conditions in Phase II and III, with Ascomycota (7.8 – 17.4%) and Basidiomycota (<5.0%) being prevalent (Figure **5.4**A). Previous studies have reported these fungal taxa enzymatically degrade residual antimicrobials in wastewater systems [191,192]. Given the presence of residual antimicrobials in PPW [10], it is plausible that similar degradation processes could have occurred in the bioreactors.

The degradable sCOD in treated PPW from the bioreactors was nearly depleted (<75 mg L^{-1} sCOD on average) prior to the hydroponic reservoirs. Additionally, the TSS in the bioreactors (algal = 7.7 – 9.6 mg L^{-1}; bacterial = ~2.4 mg L^{-1}) was higher than in all hydroponic reservoirs (0.02 – 0.15 mg L^{-1}) [100]. Therefore, the absolute abundance of heterotrophs in the hydroponic reservoirs were low compared to bioreactors despite similar relative abundance (Proteobacteria = 55.8 – 77.0%, Figure **5.3**B) and should therefore be interpreted in this context.

(A)



(B)

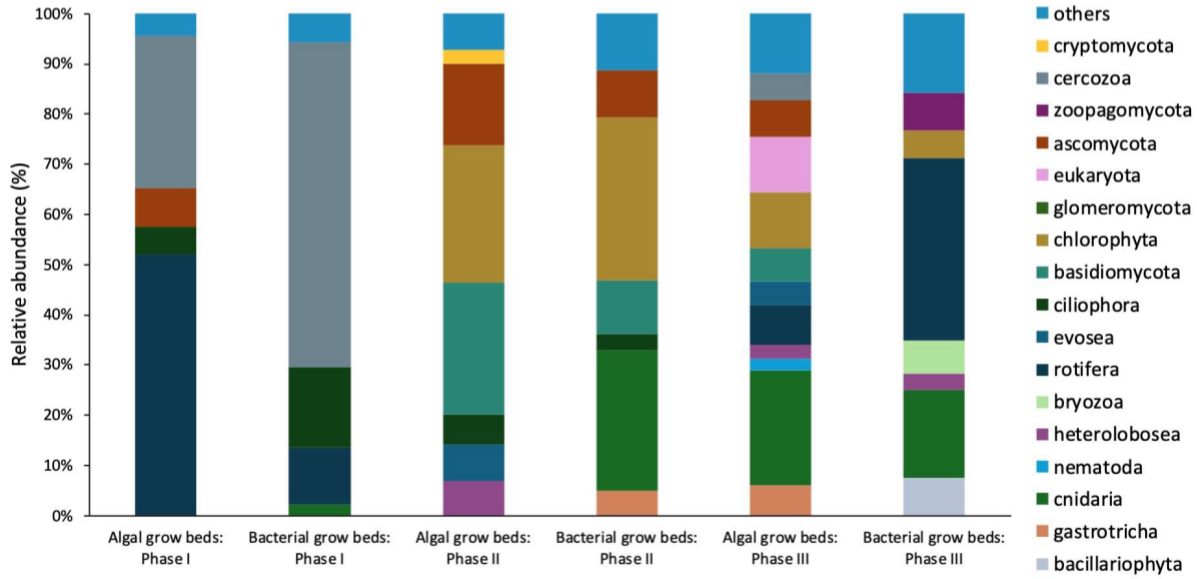


Figure 5.4: Relative 18S rRNA gene abundance at the phylum level in bioreactors (A) and hydroponic reservoirs (B) based on n=3 biological replicates. Phyla with relative abundance below 2% were assigned as others.

5.3.2 Illumination and CO_2 sparging stimulated growth of photosynthetic microorganisms in algal-bacterial treatment train

Chlorophyta (17.5%, Figure **5**.**4**A) was prevalent in the algal bioreactors, primarily consisting of *Desmodesmus*, *Chlamydomonas* and *Parachlorella* (Appendix, Table A10). Their absence in bacterial bioreactors confirms the importance of illumination and CO_2 availability for green algae proliferation (Figure **5**.**4**A). Among prokaryotes, Cyanobacteria abundance was <0.17% of prokaryotes in the algal bioreactors in Phase I and were absent in the bacteria bioreactors. Given the potential for cyanobacteria to produce toxins and off-flavor molecules [193,194], this was a favorable outcome.

Desmodesmus was the most dominant green algae genera in the algal bioreactors (9.4 – 45.8%, Figure **5**.**5**A). *Desmodesmus sp*. is a robust oleaginous microalga widely utilized for bioremediation of oily industrial wastewater, such as effluents from oil refineries or edible oil processing plants, under heterotrophic conditions [195,196]. Their proliferation in the bioreactors aligns with the high lipid content (fat, oil, and grease) in DAF effluent wastewater, which ranges from 40 to 400 mg L^{-1} [10,197]. Additionally, VFAs in PPW (~310 mg L^{-1} on average; Appendix, Table A4) could have supported the abundance of *Desmodesmus* (Appendix, Table A11) under mixotrophic conditions [198].

Upon pH control in Phase II, the relative abundance of photosynthetic organisms increased markedly in both bioreactors and downstream hydroponic reservoirs. The dominance of Chlorophyta (66.7% of eukaryotes, Figure **5**.**4**A) increased and this was accompanied by a sudden emergence of cyanobacteria in the algal bioreactors (23.7% of prokaryotes, Figure **5**.**3**A) and reservoirs (5.5% of prokaryotes, Figure **5**.**3**B). *Phormidium* was the predominant cyanobacterium in bioreactors during Phase II (8.6%, Figure **5**.**5**C).

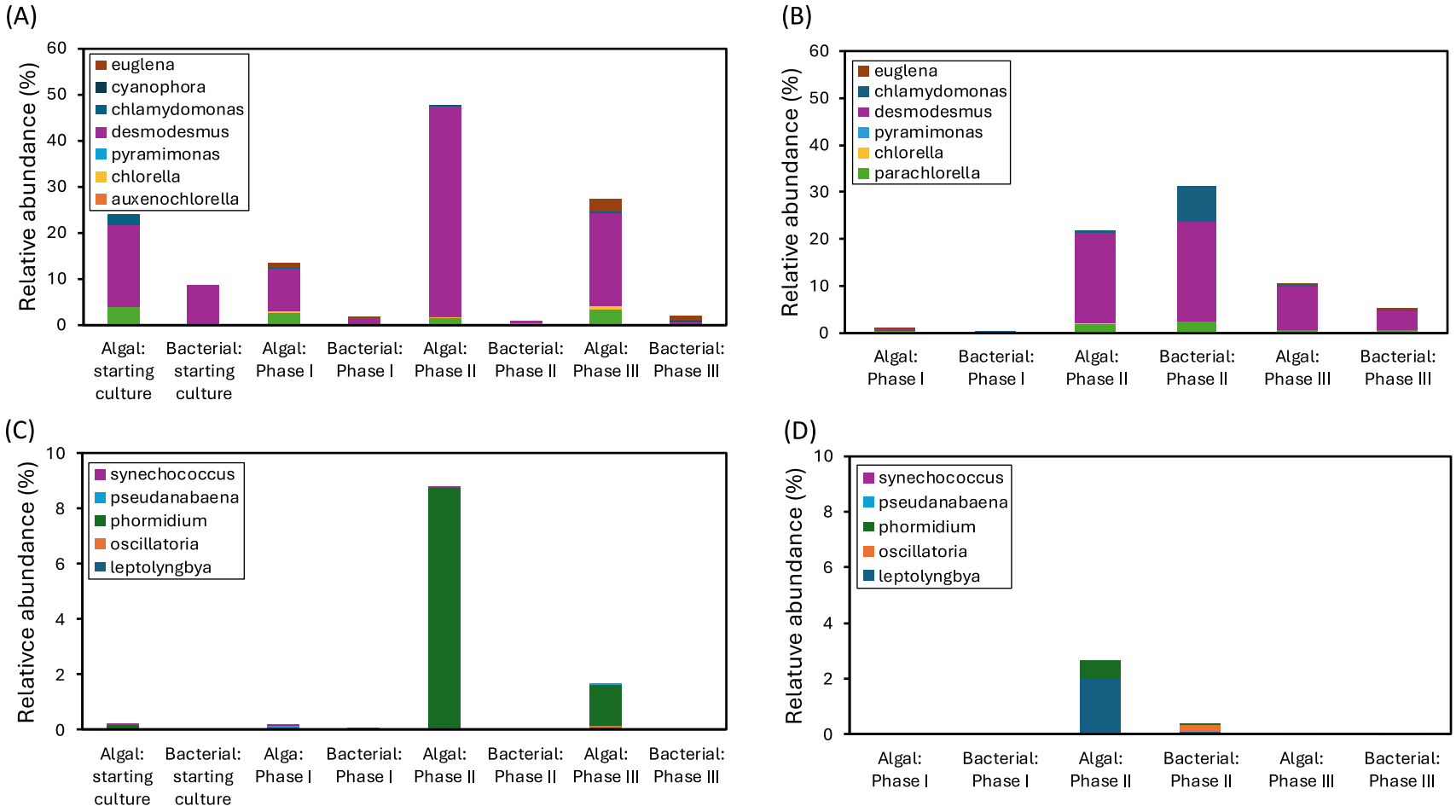


Figure 5.5: 18S rRNA of green algae (A and B) and cyanobacteria (C and D) at genus level in bioreactors and hydroponic reservoirs respectively across operational phases based on n=3 biological replicates.

Notably, *Phormidium* has been used in recent studies for treatment of agro-industrial wastewater due to its carbon sequestration and autoflocculation properties, which enhance biomass recirculation in the activated sludge process [199,200]. Unfortunately, certain strains of *Phormidium* are known to produce cyanotoxins [201].

Despite continuous reactor illumination and CO_2 sparging, a change in pH control strategy during Phase III (from a pH setpoint of 7.0 to 6.5) coincided with a marked decline in Chlorophyta (32.4%) and Cyanobacteria (1.2%). This suggests that the initial increase in photosynthetic organisms observed in Phase II may have been a transient response to pH control, followed by a restabilization of the phototrophic community under acidic conditions. In contrast, addition of CO_2 sparging in the bacterial bioreactors during Phase III did not stimulate algal growth, as the absence of illumination continued to suppress their growth. This underscores the role of illumination in sustaining photosynthetic microbial populations in Poultryponics.

5.3.3 Abundance of nitrogen cycle-related taxa in Poultryponics

The nitrifying communities in Poultryponics exhibited significant variations across the operational phases, reflecting the influence of operational conditions on nitrification performance. Nitrification rates in all of the bioreactors were low across all three phases of operation (nitrification rates < 1 mg N L^{-1} d^{-1}; NO_3-N < 2.0 mg N L^{-1}, Table **5.4**). The abundance of key AOB, (i.e. *Nitrosomonas*, *Nitrosospira*, and *Nitrosovibrio*) as well as NOB (i.e. *Nitrobacter* and *Nitrospira*) remained low in the bioreactors (<0.03%, Figure 5.6A) during baseline operation (Phase I) and upstream pH management (Phase II and III) without significant differences between bioreactor type (Figure 5.6A&B). Specifically, the abundance of genus *Nitrospira*, capable of complete ammonium oxidation to nitrate (comammox) [167] remained minimal (<0.02%, Figure

5.6A), consistent with the lack of significant nitrification in bioreactors throughout the experiment. The absence of nitrification and low nitrifier abundance could be attributed to the dominance of heterotrophs (Figure **5.3**A) and low DO (< 3.5 mg O_2 L^{-1}) to sustain nitrifying bacteria populations.

In contrast, the hydroponic reservoirs demonstrated substantially higher nitrifier abundances (up to 4.31%, Figure 5.6B) and nitrification rates (1.2 – 8.7 mg N L^{-1} d^{-1}, Table **5.4**). *Nitrosospira* (1.13%) and *Nitrosomonas* (0.57%) were dominant in the algal reservoirs while *Nitrospira* was dominant NOB in all hydroponics reservoirs (<0.98%, Figure 5.6B) even though similar levels of nitrification were observed in both systems during Phase I (Table **5.4**). The high nitrification rates observed in the hydroponic reservoirs was possible due to upstream organic matter degradation (sCOD removal = ~92%, Table **5.4**), shifting nitrification further into the hydroponic reservoirs.

During Phase II, the algal reservoirs sustained *Nitrosomonas* (0.57%) and *Nitrospira* (0.27%), which facilitated ammonium and nitrite oxidation, likely due to the continuous availability of dissolved inorganic carbon and neutral pH [71]. Similarly, the presence of algae such as *Chlorella sorokiniana* has been linked to nitrifier abundance through dissolved oxygen production in excess of saturation in marine and aquatic ecosystems [169,170] or via toxicity alleviation in high-strength wastewater treatment [46]. Our results indicate a low abundance of *Chlorella* (<1.0%) with *Desmodesmus* being the most dominant algae in the algal treatment train (9.4 – 45.8%, Figure **5.5**A&B). The proliferation of *Desmodesmus* in algal treatment train potentially alleviated VFA inhibition to nitrifying bacteria [171] under mixotrophic conditions [198], supporting high nitrifier abundance (Figure 5.6A&B).

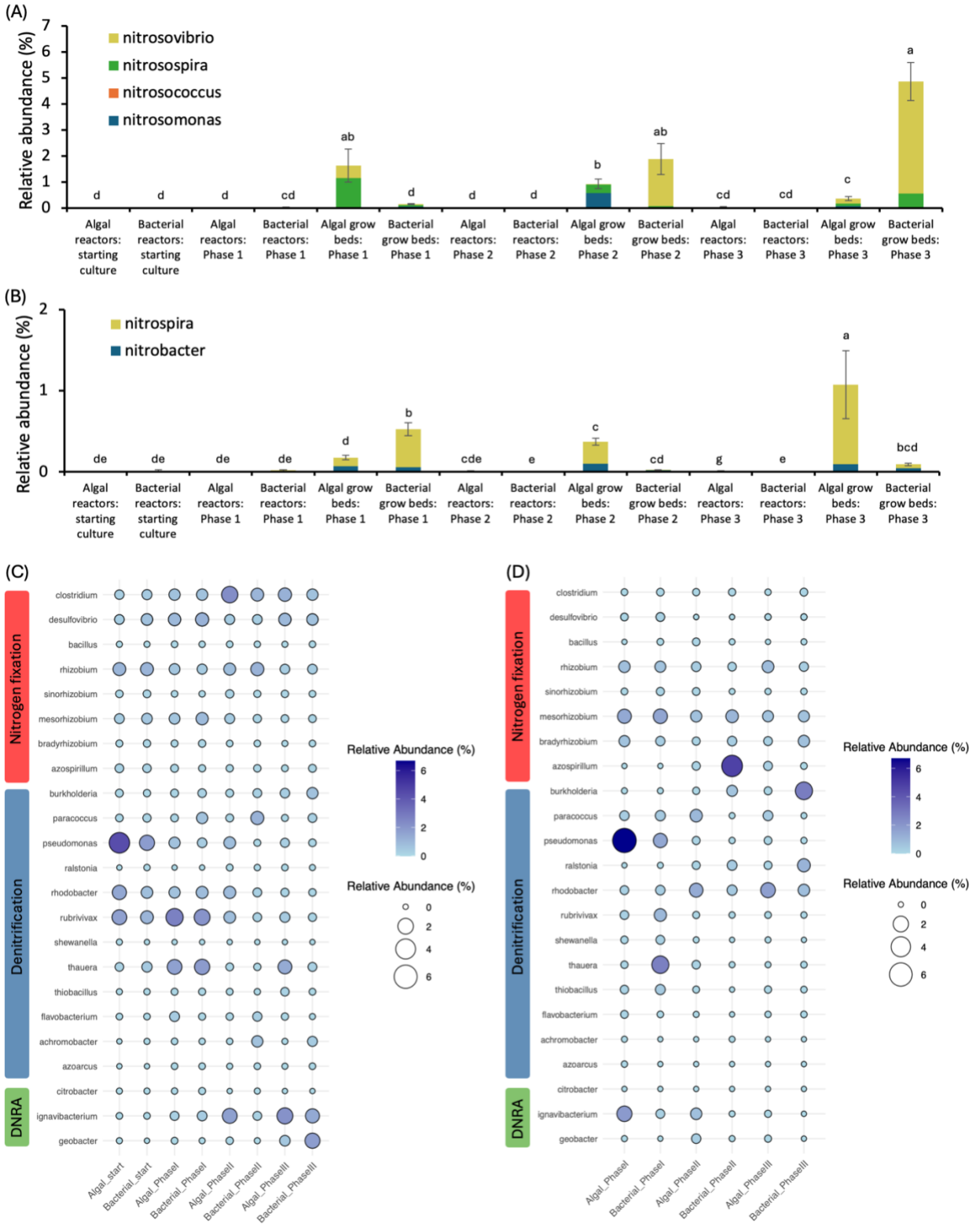


Figure 5.6: Relative abundances of ammonia oxidizing bacteria (A) and nitrite oxidizing bacteria (B) genera. Relative abundances of nitrogen-fixing, denitrifying, and dissimilatory nitrate reduction to ammonium (DNRA) genera in the bioreactors (C) and hydroponic reservoirs (D). Means (n=3) with the same letters were not statistically significant and error bars are standard deviations of total AOB and NOB abundances.

However, acid dosing during Phase II caused a significant decline in microbial diversity in the bacterial reservoirs, similar to the bioreactors, even after 98 days of steady operation under this pH control regime. The bacterial reservoirs exhibited a ~28% and ~12% reduction in richness and Shannon index respectively (Table **5**.**2**) and a sharp decline in *Nitrospira* abundance (Figure 5.6B). Consequently, nitrification rates in the bacterial reservoirs reduced by ~86% during Phase II, whereas those of the algal system had increased by ~39% compared to Phase I (Table **5**.**4**).

The increased nitrification in the algal reservoirs was consistent with the upstream CO_2 sparging, which provided carbonate buffer for pH stabilization. As a result, the algal bioreactors had achieved the same pH (7.0) as the bacterial bioreactors mostly due to dissolved CO_2 while the latter received higher H_2SO_4 dosing. Downstream in the aerated hydroponic reservoirs, CO_2 stripping in the algal system maintained neutral pH to support nitrifier activity (NO_3-N = 51.5 ± 16.6 mg N L^{-1}). In contrast, acid dosing in the bacterial system (pH = ~5.8), while optimal for lettuce cultivation [174], hampered nitrification (NO_3-N = 11.8 ± 3.8 mg N L^{-1}, Table **5**.**4**) even though the mean abundance of *Nitrosovibrio* and *Nitrosospira* increased to 1.79% and 0.09% respectively (Figure 5.6B). Given that the optimum pH for *Nitrosovibrio sp.* is between 7.7 and 7.8 [202], acid dosing during Phase II was inhibitory as mean NO_2-N concentration was ~0.1 mg N L^{-1} (Table **5**.**4**).

Phase III marked a significant recovery of nitrification in the bacterial reservoirs (Figure 5.6B), with increases in the abundance of *Nitrosospira* (0.56%) and *Nitrosovibrio* (4.31%). This was consistent with the ~3.4-fold increase in nitrification rates similar to that of the algal system (~6.2 mg N L^{-1} d^{-1}, Table **5**.**4**). Although pH stabilization via CO_2 sparging favored ammonium oxidizers (*Nitrosospira* and *Nitrosovibrio*), upstream treatment in bioreactors and continuous

aeration in the reservoirs also supported the improved nitrification rates (~6.1 mg N L^{-1} d^{-1}, Table **5.4**) even at low NOB abundances; *Nitrobacter* (0.04%) and *Nitrospira* (0.05%).

Other nitrogen cycling pathways including nitrogen fixation, denitrification, and dissimilatory nitrate reduction to ammonium (DNRA) significantly influenced nitrogen retention and loss in Poultryponics. In the bioreactors, anoxic conditions due to high biomass growth promoted denitrifiers such as *Thauera*, *Rubrivivax*, *Rhodobacter* and *Pseudomonas* (Figure 5.6C). Similarly, DNRA-related taxa such as *Ignavibacterium* and *Geobacter* were also prominent in the bioreactors, indicating their role in ammonium retention under high C/N ratio [172,173]; 12 – 15 on average in Poultryponics [100].

The trends of nitrogen retention and loss in Poultryponics mirrored shifts among denitrifiers and DNRA-related taxa. Denitrification was highest in Phase I (19 – 24% of TN) in both systems (Table **5.4**), coinciding with the high relative abundances of *Thauera* (1.77%), *Rubrivivax* (2.42%), and *Pseudomonas* (0.51%, Figure 5.6C) and low NO_3-N concentrations in the bioreactors (Table **5.4**). Denitrification reduced to 3.2% in algal and 0.8% in bacterial bioreactors, correlating with a shift toward DNRA in Phase II. Acid dosing likely contributed to stronger denitrification inhibition particularly in bacterial bioreactors where *Thauera* and *Rubrivivax* decreased sharply (<0.25%), while *Ignavibacterium* (1.72%) and *Geobacter* (1.80%) increased (Figure 5.6C), favoring high ammonium retention (NH_4–N concentration = 58 – 64 mg N L^{-1}, Table **5.4**). Similarly, denitrification reduced in the bacterial reservoirs (2.8%) compared to the algal system (9.8%, Table **5.4**) coinciding with the reduction of denitrifier abundance (*Pseudomonas* = 0.14% and 0.02%; *Rubrivivax* = 0.06% and 0.02%; and *Thauera* = 0.19% and 0.01% in algal and bacterial reservoirs respectively, Figure 5.6D). By Phase III, denitrification resurged in bacterial system (Table **5.4**) as denitrifiers partially recovered (*Rhodobacter* = 0.79%;

Thauera = 0.24%; *Pseudomonas* = 0.10%, Figure 5.6D). Meanwhile, DNRA taxa remained relatively high in both bioreactors, indicating ongoing competition between nitrogen retention and loss in Poultryponics.

5.3.4 Indicators of plant growth-promotion in Poultryponics

A notable observation in our pilot-scale study was that nitrogen, phosphorus and iron concentrations in lettuce leaf tissues (7.1%, 1.2%, and 167 mg kg^{-1} respectively) irrigated with treated PPW were comparable to the those irrigated with commercial hydroponic fertilizer [174]. This is remarkable given the low concentrations of iron observed in the treated wastewater (<0.25 mg L^{-1}; Table **5.5**) compared to the typical levels in hydroponic solutions; >2 mg L^{-1} [73].

The hydroponic reservoirs supported several putative siderophore-producing bacteria, dominated by *Pseudomonas*, *Azospirillum* and *Sphingomonas* (Figure 5.7). Siderophores are iron-chelating molecules, typically secreted by bacteria, that can improve iron availability to plants [179]. This may help explain why plants were not iron deficient despite low iron concentrations in PPW [174]. The average siderophore unit was higher in the algal system (23.7% in bioreactors and 20.0% in reservoirs) compared to the bacterial system (18.2% in bioreactors and no detection in reservoirs, Table **5.6**). Consequently, the elevated siderophore activity in the algal system (~0.6 ng L^{-1} deferoxamine mesylate equivalence) correlated with high relative abundance of siderophore-producing genera such as *Pseudomonas*, *Azospirillum* and *Sphingomonas* [179]; with peak average abundances of *Sphingomonas* reaching 3.5% in the algal system compared to 1.5% in the bacterial system (Figure 5.7). The high abundance of *Sphingomonas* in the hydroponic reservoirs also suggest a potential role in limiting pathogens due to its competitive iron acquisition mechanisms [203], and aligns with the non-detection of *Salmonella*, *E. coli*, and *Campylobacter* in lettuce

cultivated in Poultryponics across all phases [204]. Notably, some fungal and oomycete taxa known to cause grey mold and root rot in lettuce such as *Ulocladium sp*. and *Pythium sp*. [205,206] were present in the hydroponic reservoirs, with higher relative abundances in the bacterial system (Appendix, Table A15). However, the comparable lettuce growth performance in both systems [174] suggests that these taxa were either non-pathogenic under the operational conditions or that their effects were suppressed by other microbial antagonism.

Table 5.5: Concentration of iron in poultry processing wastewater, treated effluents and lettuce leaf tissue.

Fe concentration in water samples (mg L^{-1})				
Influent PPW	Treated effluent			
0.50 ± 0.04	Algal-bacteria train		Bacterial train	
	0.24 ± 0.11 [a]		0.21 ± 0.01 [a]	
Fe concentration in leaf tissue (mg kg^{-1})				
Operation	Treatment			
	Algal	Bacterial	Supplemental	Control
Phase I	183 ± 21 [a]	190 ± 48 [a]	165 ± 23 [a]	129 ± 16 [a]
Phase II	216 ± 129 [a]	330 ± 23 [a]	279 ± 25 [a]	215 ± 10 [a]
Phase III	342 ± 69 [a]	296 ± 78 [a]	402 ± 105 [a]	156 ± 21 [b]

Values are means ± standard deviation of 3 biological replicates. Within each treatment type, means with similar letters are not statistically different based on the Tukey's HSD test ($p>0.05$).

In addition to siderophores, many plant-associated bacteria produce plant hormones that affect plant growth, architecture, and pathogen resistance. Untargeted LC-MS/MS analysis identified plant growth-promoting compounds; azelaic acid, trans-3-indoleacrylic acid (trans-IAA), and decanamide in the hydroponic reservoirs with match factors >90% (Table **5.7**). Azelaic acid is known to induce systemic resistance against pathogens [207], while trans-IAA acts as a plant

auxin, influencing root architecture. Although decanamide was detected, the reported effect of stimulating root hair elongation and altering root architecture is attributed to N-isobutyl decanamide, a structurally related but distinct compound [208].

While trans-IAA was detected during the untargeted analysis, it did not match the retention time and MS/MS spectra under the spiked/targeted approach, suggesting either its absence or presence at concentrations below detection thresholds (Table **5**.**7**). Conversely, azelaic acid and decanamide were detected based on retention time and MS/MS spectral matches, but at concentrations below their respective limits of detection (LOD). Results from the cytokinin bioassays similarly indicated little to no detectable cytokinin activity in the treatment samples (Figure **5**.**8** and Figure **5**.**9**). The detection of these growth-promoting compounds, albeit at low concentrations, suggests they may be present but potentially diluted within the hydroponic reservoirs, rapidly degraded, affected by pH, or were constrained by environmental conditions or metabolic interactions within the hydroponic reservoirs.

Overall, these findings highlight that siderophore activity, and the presence of plant growth-promoting metabolites in the hydroponic reservoirs may have contributed to enhanced nutrient bioavailability, pathogen suppression, and plant resilience in Poultryponics.

genus	Algal: Phase I	Bacterial: Phase I	Algal: Phase II	Bacterial: Phase II	Algal: Phase III	Bacterial: Phase III
azospirillum	0.0 ± 0.0%	0.0 ± 0.0%	0.2 ± 0.1%	4.8 ± 2.4%	0.3 ± 0.3%	0.0 ± 0.0%
bacillus	0.0 ± 0.0%	0.0 ± 0.1%	0.1 ± 0.1%	0.0 ± 0.0%	0.0 ± 0.0%	0.0 ± 0.0%
kosakonia	0.0 ± 0.0%	0.0 ± 0.0%	0.0 ± 0.0%	0.0 ± 0.0%	0.0 ± 0.0%	0.0 ± 0.0%
sphingomonas	0.8 ± 0.2%	1.8 ± 0.4%	0.5 ± 0.2%	0.6 ± 0.1%	3.5 ± 4.1%	1.5 ± 0.0%
paenibacillus	0.0 ± 0.0%	0.0 ± 0.0%	0.0 ± 0.0%	0.0 ± 0.0%	0.0 ± 0.0%	0.0 ± 0.0%
pseudomonas	6.7 ± 2.9%	1.5 ± 0.9%	0.1 ± 0.4%	0.0 ± 0.0%	0.1 ± 0.1%	0.0 ± 0.0%
rhodococcus	0.0 ± 0.0%	0.0 ± 0.0%	0.0 ± 0.0%	0.0 ± 0.0%	0.0 ± 0.0%	0.0 ± 0.0%
streptomyces	0.0 ± 0.0%	0.0 ± 0.0%	0.0 ± 0.0%	0.0 ± 0.0%	0.0 ± 0.0%	0.0 ± 0.0%
pantoea	0.0 ± 0.0%	0.0 ± 0.0%	0.0 ± 0.0%	0.0 ± 0.0%	0.0 ± 0.0%	0.0 ± 0.0%
Total	7.5 ± 2.2% [a]	3.4 ± 0.7% [ab]	0.9 ± 0.2% [b]	5.4 ± 1.6% [a]	3.9 ± 1.1% [ab]	1.6 ± 0.5% [ab]

scale
0.0%
<2.0%
<4.0%
>4.0%
>6.0%

Figure 5.7: Relative abundance of siderophore-producing bacteria in the hydroponic reservoirs across operational phases. Color intensity of scale indicates the relative abundance of each genus relative to the total number of sequences in each sample based on n=3 biological replicates.

Table 5.6: Quantitative analysis of siderophore production in poultry processing wastewater and treated effluents during Phase III

	OD630	Percent siderophore unit (psu)	Concentration (ng L^{-1} Deferoxamine mesylate equivalence)
Raw poultry processing wastewater	0.41 ± 0.04^{b}	$31.6 \pm 6.6\%^{a}$	1.07 ± 0.53^{a}
Algal bioreactors	0.45 ± 0.01^{b}	$23.7 \pm 1.8\%^{b}$	0.57 ± 0.03^{b}
Bacterial bioreactors	0.49 ± 0.01^{b}	$18.2 \pm 2.6\%^{c}$	0.39 ± 0.03^{c}
Algal reservoirs	0.47 ± 0.04^{b}	$20.0 \pm 6.9\%^{b}$	0.50 ± 0.23^{b}
Bacterial reservoirs*	0.66 ± 0.06^{a}	-	-

Values are the means ± standard deviation of 3 biological replicates
* The OD 630 value was not different than the blank thus, psu and concentration could not be calculated
Means with the same letters were not statistically significant based on Tukey HSD ($p>0.05$).

Table 5.7: Confirmation of plant growth-promoting compounds in water samples from hydroponic reservoirs during Phase III

Compound	Function	Match factor (mzcloud)	RT and MS/MS match	LOD (μg L^{-1})	LOQ (μg L^{-1})	Algal-bacterial reservoirs (μg L^{-1})	Bacterial reservoirs (μg L^{-1})
Azelaic acid	Indicator for lipid peroxidation in plants and induces systemic resistance to pathogens	99.8%	Yes	10.8	32.6	2.8 ± 0.4^{a}	2.6 ± 0.3^{a}
trans-3-Indoleacrylic acid	Plant auxin	90.6%	No	1.6	5.0	nd	nd
Decanamide	Alters root architecture by stimulating root hair elongation	97.5%	Yes	2.0	6.0	1.0 ± 0.5^{a}	1.1 ± 0.6^{a}

Values are the means ± standard deviation of 3 biological replicates. Means with the same letters were not statistically different between the algal and bacterial systems based on Tukey HSD ($p>0.05$).
LOD: Limit of detection; LOQ: Limit of quantification; nd: not detected

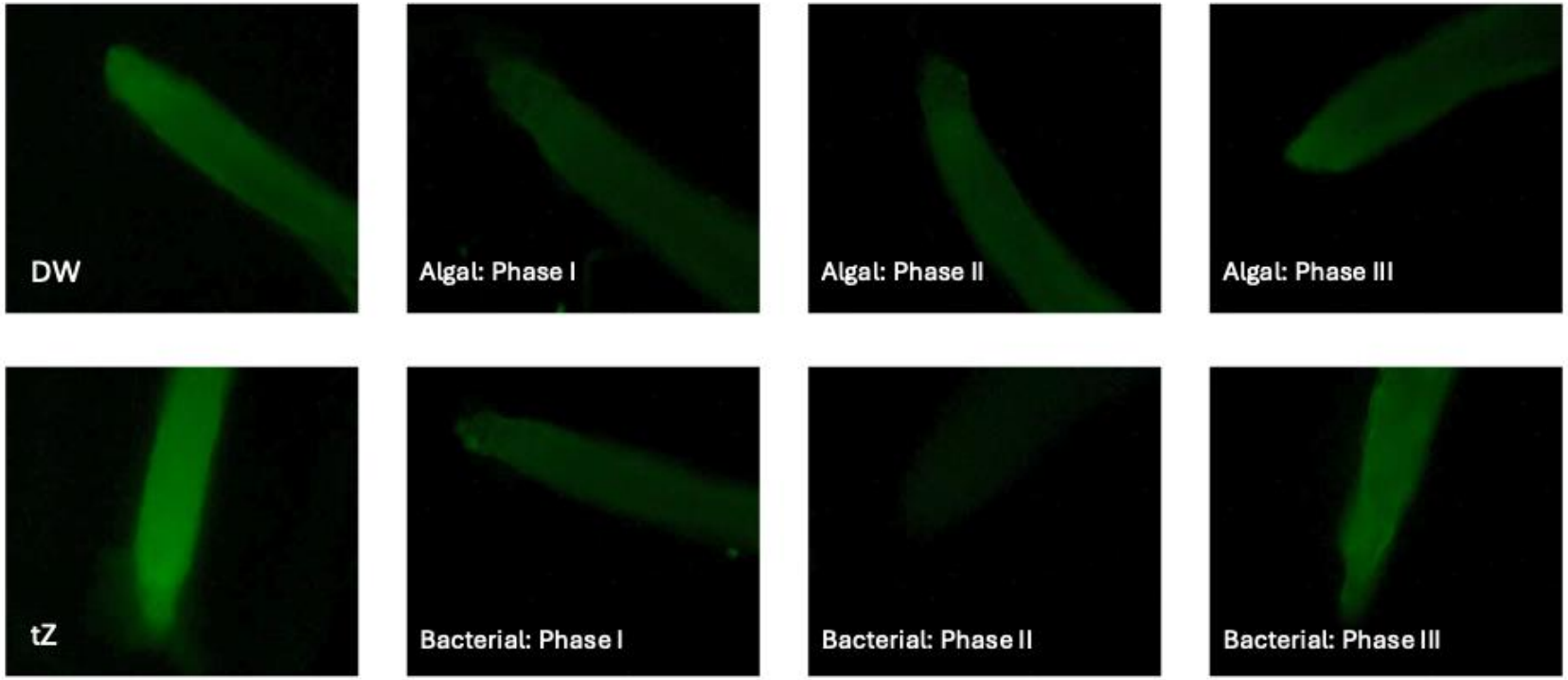


Figure 5.8: GFP fluorescence in *Arabidopsis thaliana* TCSn:GFP reporter line following 24-hour treatment with Poultryponics water extracts from hydroponic reservoirs across operational phases. Distilled water (DW) and 1 μM *trans*-zeatin (tZ) served as negative and positive controls, respectively.

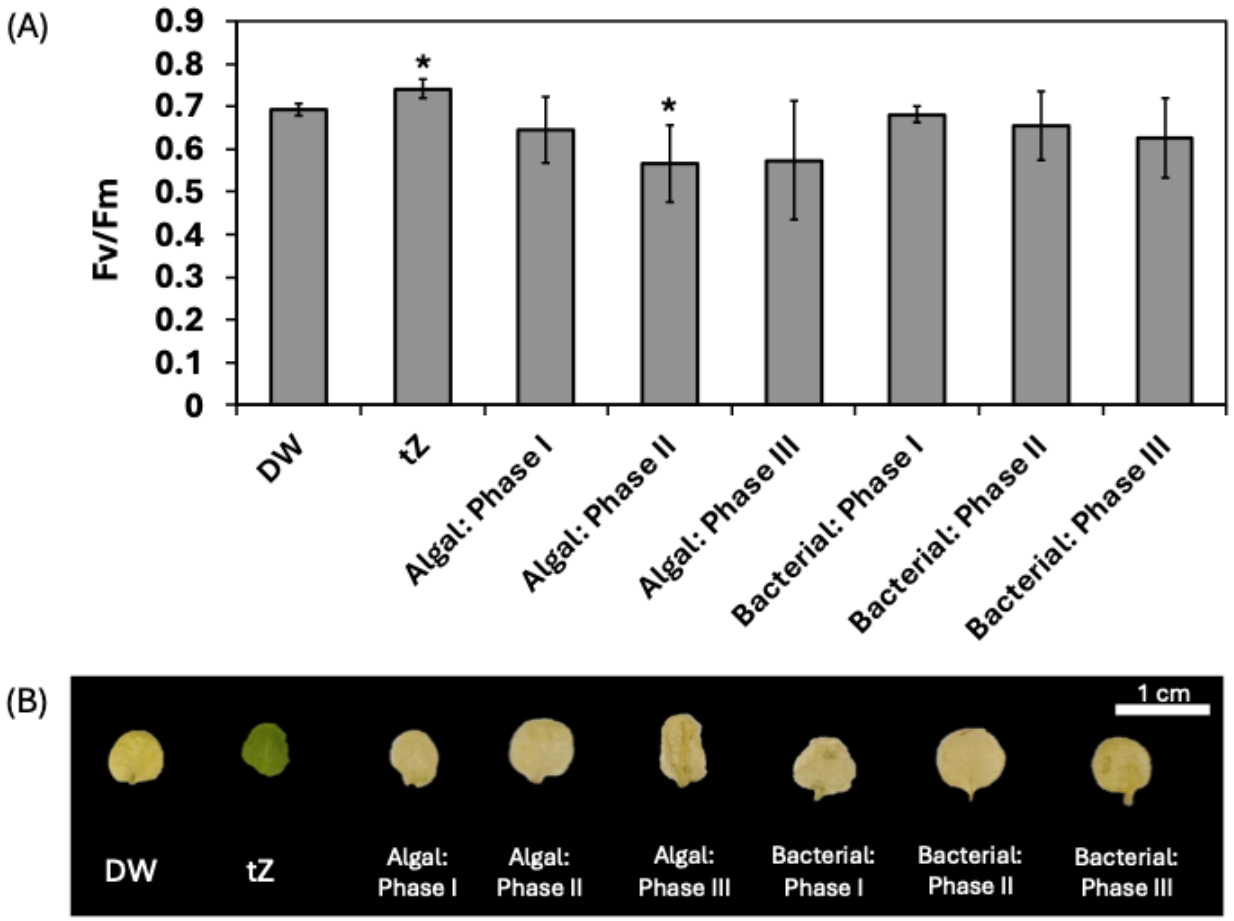


Figure 5.9: Photosystem II efficiency (Fv/Fm) of *Arabidopsis thaliana* leaves at 144 hours after dark-induced senescence assay, following treatment with Poultryponics water extracts from hydroponic reservoirs across operational phases (A). Representative images of *Arabidopsis leaves* after 144-hour dark-induced senescence assay (B). DW and tZ were used as negative and positive controls, respectively. Error bars represent standard error; asterisks (*) indicate significant differences relative to DW ($p < 0.05$).

5.3.5 Microbial taxa impacting food safety in hydroponic reservoirs

Cyanobacteria was notable in the algal reservoirs during Phase II (5.5%, Figure **5**.**3**B) and bacterial reservoirs (2.4%, Figure **5**.**3**B), likely supported by CO_2 sparging and slightly neutral pH [209]. While the specific contributions of cyanobacteria to nutrient dynamics in Poultryponics remain unclear, the cyanobacterial genera identified in the hydroponic reservoirs (*Synechococcus*, *Pseudanabaena*, *Phormidium*, *Oscillatoria*, and *Leptolyngbya,* Figure **5**.**5**C&D) are known producers of cyanotoxins, including microcystins, anatoxin-a, and beta-methylamino-L-alanine (BMAA) [194], which may potential accumulate in hydroponic vegetables (i.e. lettuce). However, the LC-MS/MS analysis of water samples in the hydroponic reservoirs did not detect any of these cyanotoxins, suggesting their absence or presence below detection thresholds.

Fungal taxa associated with organic matter degradation and nutrient cycling, such as Basidiomycota (6.5%) and Ascomycota (7.1%) persisted during Phase III (Figure **5**.**4**B). Notably, *Aspergillus flavus*, *Rhizopus oryzae*, and *Penicillium sp*. were the dominant fungal species with known implications for food quality and safety (Appendix, Table A15). *A. flavus* is a well-known producer of aflatoxins, a potent group of carcinogenic mycotoxins that can accumulate in crops, posing significant risks to human health [210]. Meanwhile, *R. oryzae*, though not a toxin producer, is a common spoilage fungus that accelerates post-harvest decay of fresh produce through rapid colonization and enzymatic degradation of plant tissues, reducing the shelf life of hydroponic vegetables [211].

Furthermore, Chlamydiae contributed significantly ($p<0.05$) to community dissimilarity particularly in the bacterial hydroponic reservoirs where its relative abundance reduced from 12.3% in Phase I, 2.4% in Phase II and not detected in Phase III (Figure **5**.**4**B). Chlamydial species, such as *Chlamydia psittaci*, and *Chlamydia gallinacea*, are commonly associated with the poultry

industry, where they colonize the respiratory tracts of birds and can be shed into wastewater through fecal matter or respiratory secretions [212]. Their high initial relative abundance during Phase I suggests potential carryover from PPW. Given the potential risks posed by these taxa, a comprehensive risk assessment is required to evaluate spoilage risks, post-harvest quality, and mycotoxin production to ensure safe and sustainable bioponic production.

5.4 Conclusion

Microbial community dynamics in Poultryponics were strongly influenced by illumination, pH control, and CO_2 buffering. In the bioreactors, heterotrophic activity was dominant, where Proteobacteria (46.3–83.0%), Bacteroidetes (6.5–20.6%) and fungal decomposers (Ascomycota and Basidiomycota) facilitated organic matter degradation (>80% sCOD removal) and ammonification. Illumination and CO_2 supplementation in algal bioreactors supported Chlorophyta (<66.7%), particularly *Desmodesmus* (<45.8%). Nitrification was limited in the bioreactors due to low nitrifier abundances (<0.03%) and competition with heterotrophs across all operational phases. However, pH-modulating effects of CO_2 sparging increased nitrifier abundance (<4.31%) in hydroponic reservoirs, supporting nitrification rates of 6.3 – 8.7 mg N L^{-1} d^{-1}. Acid dosing hampered denitrification while favoring DNRA, reflecting nitrogen retention/loss trade-offs. The hydroponic reservoirs exhibited siderophore activity (<0.6 ng L^{-1} deferoxamine mesylate equivalence) and low levels of plant growth-promoting metabolites. However, the presence of cyanobacterial and fungal taxa in Poultryponics raises concerns regarding toxin accumulation, post-harvest spoilage, and food safety risks, necessitating further research to assess their impacts on hydroponic produce.

Chapter 6: Pilot-scale evaluation of sequential bioreactor configuration in Poultryponics: Impact of system design on organics removal, nitrogen transformation and microbial communities

Pilot-scale evaluation of sequential bioreactor configuration in Poultryponics: Impact of system design on organics removal, nitrogen transformation and microbial communities

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Highlights

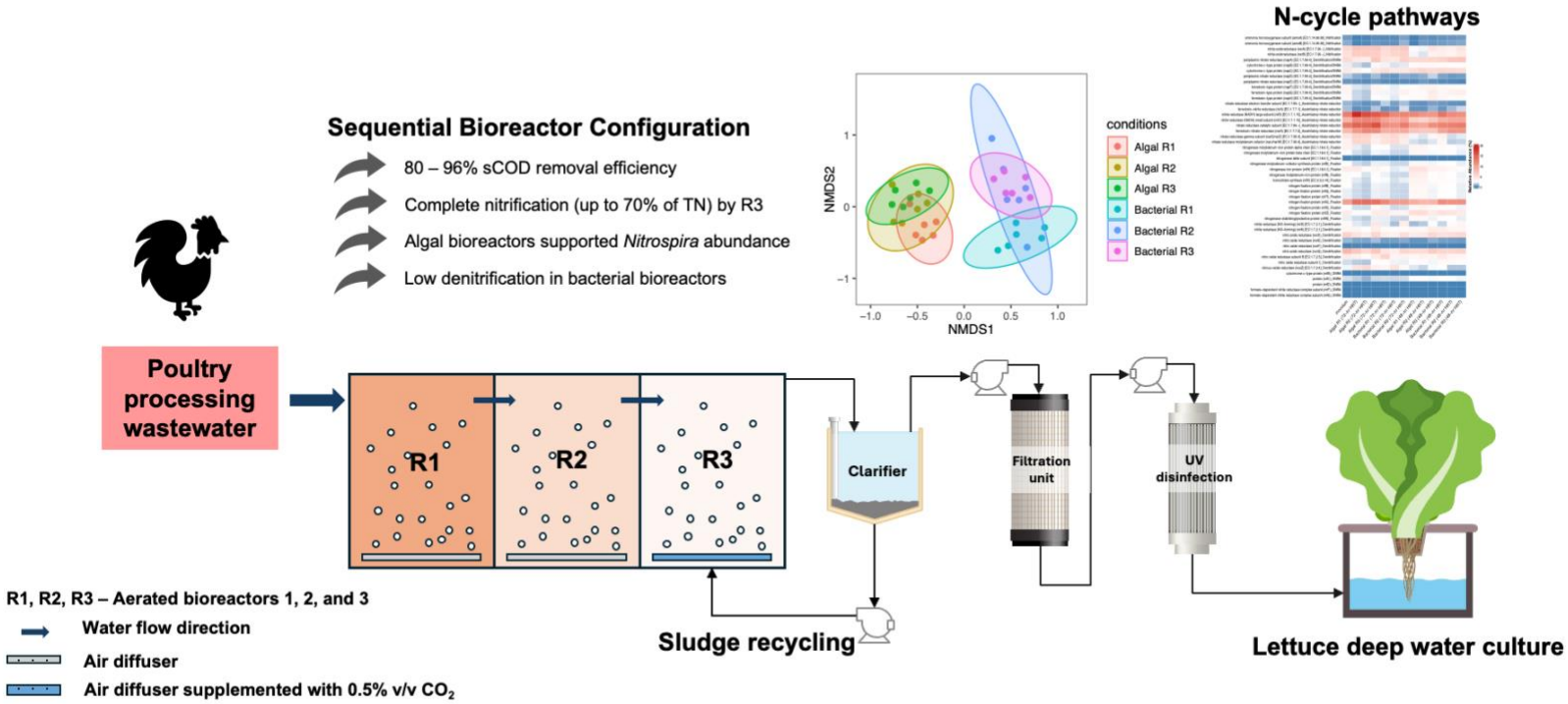
1. Complete nitrification was achieved in series bioreactors at 72-hr HRT
2. 48-hr HRT doubled loading rates and reduced nitrification performance
3. Algae supported nitrite oxidation during high loading rates
4. Persistence of *Limnothrix* raises food safety concerns

Abstract

Poultryponics is a pilot-scale integrated system that recycles poultry processing wastewater (PPW) for hydroponic crop irrigation. This study evaluated the effects of bioreactor sequencing and hydraulic retention time (HRT) on organics removal, nutrient transformation and microbial dynamics over 310 days of continuous operation. The system treated ~115 L d^{-1} PPW using two parallel trains (algal vs. bacterial), each comprising three bioreactors in series (R1 – R3). Total bioreactor residence time was tested at 72-hr HRT and 48-hr HRT. At 72-hr HRT, both algal and bacterial bioreactors achieved high sCOD removal (80 – 96%) and sustained nitrification, with early-stage nitrification in algal R1 supported by elevated DO (~6.0 mg L^{-1}) and *Desmodesmus* enrichment (11–27%). Reducing the HRT to 48 hrs doubled organic and nitrogen loading, suppressing nitrite oxidation in bacterial reactors, while complete nitrification persisted in algal R3, aided by *Nitrospira* abundance (<1.7%). Nitrogen loss peaked at 43% of total nitrogen (TN) in algal bioreactors under the 48-hr HRT, coinciding with the proliferation of denitrifiers such as *Zoogloea* and *Hydrogenophaga*, and the presence of carbon sources like VFAs, amino acids, and algal metabolites. Although treated PPW contained high levels of NO_3-N (~70% of TN), elevated Na^+ (140 – 193 mg L^{-1}) and low K^+ (54 – 81 mg L^{-1}) could induce salt stress and nutrient imbalance in downstream hydroponic crops. The presence of toxin-producing cyanobacteria such as *Limnothrix* raises potential food safety concerns. Overall, these results demonstrates that series bioreactor configuration enables upstream organics removal and complete nitrification, offering operational flexibility for optimized hydroponic management.

Keywords: Bioponics; Poultry processing wastewater; Nitrification; Denitrification; Wastewater treatment

Graphical abstract



6.1 Introduction

Global poultry meat production continues to rise, with estimates reaching 104 million tonnes annually [53]. In the poultry slaughtering and meat processing industry alone, approximately 901 billion liters of wastewater are projected to be generated globally in 2025, with an estimated treatment cost of $953 million [8]. Poultryponics has emerged as a sustainable approach to repurpose nutrients in poultry processing wastewater (PPW) for hydroponic food crop production [100]. While past reuse efforts have focused on non-food applications due to concerns over pathogen contamination [12], poultryponics has demonstrated that PPW can safely support hydroponic lettuce cultivation even under extreme contamination scenarios, without detectable pathogen presence [204].

Nutrient transformation in poultryponics depends on several microbial processes. Initially, organic compounds in PPW such as fats, proteins, amino acids [10,213] are anaerobically mineralized to volatile fatty acids (VFAs) and ammonium-N. For plant uptake, ammonium-N must be converted to nitrate-N via nitrification; a two-step aerobic process mediated by ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) [39]. However, nitrification is often suppressed in high-strength wastewaters like PPW due to competition for dissolved oxygen (DO) with fast-growing heterotrophs [67,100] and the inhibitory effects of organic intermediates such as VFAs [171].

In our previous pilot-scale study, bioreactors achieved >80% organic removal, however, nitrification was inhibited due to low DO (<3.5 mg L^{-1}) and short hydraulic retention time (HRT) of 24 hrs, resulting in nitrification occurring downstream in the hydroponic plant reservoirs [100]. This outcome presented operational challenges due to the incompatible pH requirements between nitrifying bacteria (7.0 – 8.0) [85,86] and lettuce plants (5.0 – 6.5) [73], which hindered nutrient optimization and lettuce growth in hydroponic reservoirs [174].

Conventional biological wastewater treatment systems use multistage bioreactors configurations to manage high-strength wastewaters by compartmentalizing microbial processes [214,215]. These systems promote efficient organic removal and nitrification even under high loading conditions and reduced HRTs while reducing operational footprint [216,217]. Translating this strategy to poultryponics could optimize organics mineralization and nitrogen transformation upstream of the hydroponic reservoirs, thereby enabling strategic pH control and nutrient supplementation that optimizes plant production.

Moreover, recent studies have demonstrated that incorporating green algae into wastewater treatment improves system performance by supplying DO [169,170], thereby supporting heterotrophic and autotrophic microbial communities involved in organics degradation [46] and nitrification [43,47]. However, dense algal biomass can also promote floc formation and internal oxygen gradients that support localized anoxic microenvironments[218,219], enabling nitrogen loss via denitrification[220], particularly when VFAs or other carbon sources are present [221]. In the context of poultryponics, processes that enhance nitrification while minimizing losses of N to denitrification are desired. Prior studies suggest that the presence of algae may improve nitrification but also increase nitrogen loss from the system [174]. However, the role of algal-bacterial interactions on nitrogen dynamics (retention/loss) remains understudied in recent bioponic studies [25,65,127,222,223], limiting our understanding of the impacts of long-term microbial succession, and operational fluctuations typical of commercial-scale applications.

Therefore, the objectives of this study were to: (a) implement a multistage bioreactor configuration (series setup) to promote organics removal and complete nitrification, thereby decoupling nutrient transformation from the hydroponic reservoirs; (b) evaluate the impact of bioreactor HRT and the presence of algae on organics removal and nitrogen transformation; and

(c) elucidate the dynamic changes between key microbial taxa (algae, nitrifiers and denitrifiers) and their relationships with nitrogen dynamics under long-term continuous operation (>300 days) at pilot-scale using real poultry processing wastewater. Although lettuce was cultivated in the hydroponic grow beds of the system, the focus of the present study was on the wastewater treatment functions of the system. Lettuce growth in the system is the subject of a future article.

6.2 Materials and Methods

6.2.1 Reactor configuration and operation of Poultryponics

The schematic representation of the series bioreactor configuration and other components of the pilot scale Poultryponics system is shown in Figure **6.1**. PPW was continuously pumped into the two parallel treatment trains. Each train consisted of three bioreactors in series (~57 L maximum working volume each), a ~57 L clarifier (TAMCO industries, USA), a 1μ bag filter (PENTAIR, ES31NA410), a UV disinfection unit (HQUA–OWS–12), and a ~114 L holding tank (TAMCO industries, USA) that was used to irrigate the deep-water plant grow beds (Appendix, Figure A4). The bioreactors were constructed from a sealable ~76 L glass aquarium. Each aquarium was modified by drilling and installing two outlet ports fitted with ball valves to allow for HRT adjustment without changing the influent flow rate (~57 L d^{-1}). The bioreactors were designed such that the effluent from one reactor flowed sequentially into the next in the series, with the final effluent from the third bioreactor draining into the clarifier.

In train 1, each bioreactor was illuminated using LED lamps (~290 μmol m^{-1} s^{-1}, 12h:12h light-dark cycle) to support algae growth and photosynthesis and are subsequently referred to as the algal bioreactors, although in reality it was a mixture of algae and bacteria. In train 2, the bioreactors were covered with a dark cloth to suppress photosynthesis and algae growth and are referred to as the bacterial bioreactors. All the bioreactors were sparged with 300 mL min^{-1} of air. Additionally, the third bioreactor in each treatment train received supplemental CO_2 (0.5% v/v in air) as an inorganic carbon source to enhance nitrifier abundance and support autotrophic nitrification. Each bioreactor was outfitted with a pH controller (Milwaukee, MC122, USA), oxidation-reduction potential (ORP) probe (Milwaukee, MC122, USA), DO probes (Mettler Toledo InPro System), and light/temperature data loggers (HOBO, MX2202, USA).

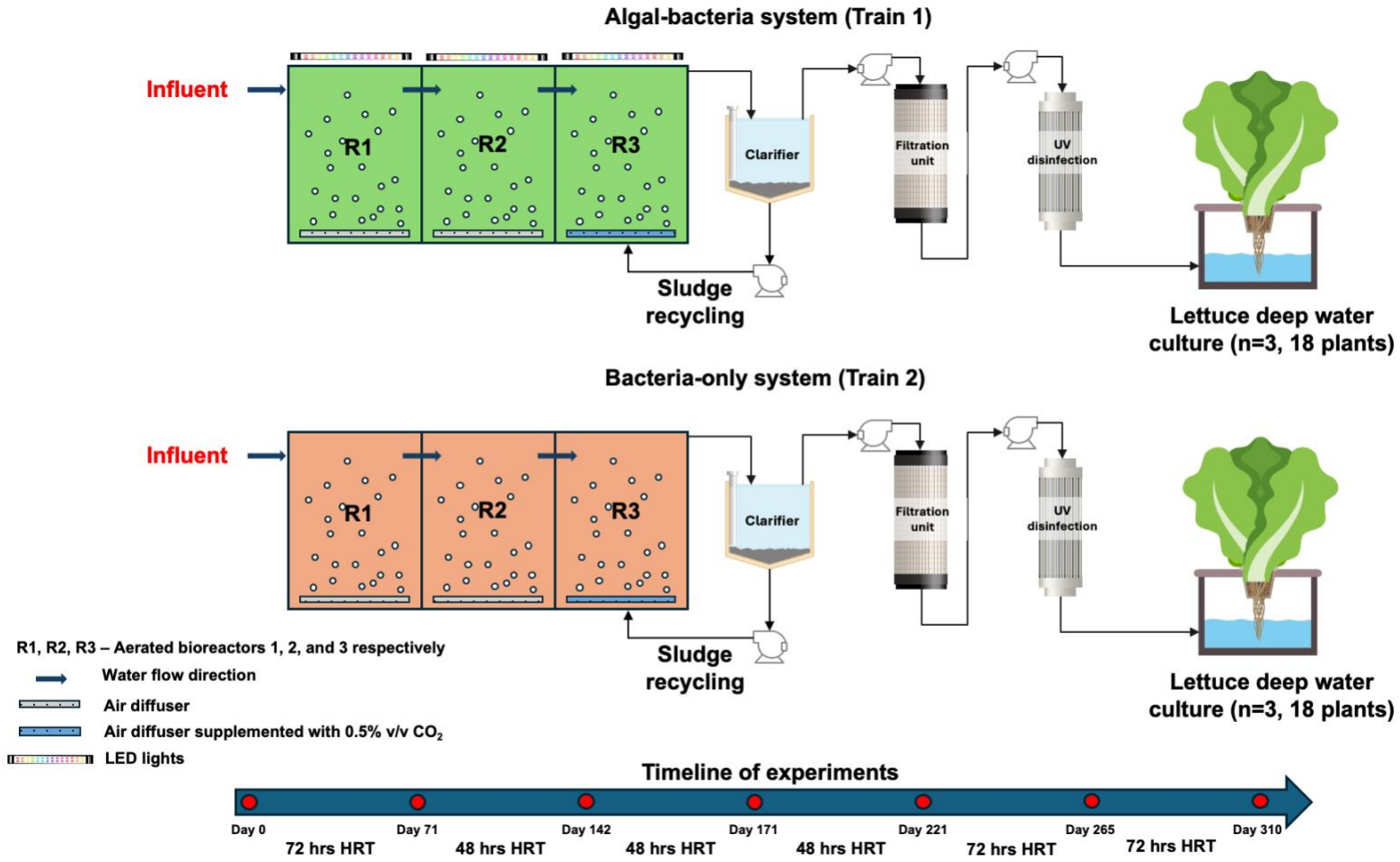


Figure 6.1: Schematic representation of the pilot-scale sequential bioreactor configuration in Poultryponics. Bioreactors in Train 1 received illumination to promote algae whiles bioreactors in Train 2 were covered to suppress algae. Treated effluents from each train irrigated lettuce in a deep-water culture. The biological replicates of experiments were conducted under the two HRTs (72 hrs and 48 hrs).

Effluents from the third bioreactors in each train flowed into the clarifier, where solids were settled and periodically returned to the third bioreactors to enhance solids retention and promote nitrification [224]. The solids retention time (SRT) in the third bioreactors was estimated at 57 and 27 days by the end of the study for the algal-bacteria and bacteria-only bioreactors, respectively, due to continuous sludge recycling from the clarifiers. The clarified supernatant was passed through a 1 μm bag filter and a UV disinfection unit (~1 h HRT each) before temporary storage in the holding tank. Treated effluents were pumped into an indoor deep-water culture hydroponic system for lettuce cultivation. Each treatment train irrigated three independent reservoirs (~37.9 L), each fitted with drip emitters and a floating polystyrene board containing 6 precut holes for planting butterhead lettuce (18 plants per treatment). The hydroponic system was maintained under controlled environmental conditions, with full-spectrum LED lighting (640 mmol photons m^{-2} s^{-1} on an 8h:16h light-dark cycle, Volt Lighting, VGL2-750-L, USA) and temperature range of 20–25°C.

6.2.2 Seed culture and wastewater source

The bioreactors were inoculated with a mixed algal–nitrifying bacterial consortium from a biofloc aquaculture system at Auburn University. An initial biomass concentration of 1.4 ± 0.1 g L^{-1}; as mixed liquor suspended solids (MLSS), was established uniformly across all bioreactors. The systems were operated for 40 days prior to lettuce cultivation to allow for development and acclimation of microbial communities to PPW.

PPW used in this study was collected from a commercial broiler processing facility in the southeastern United States, which processes ~642,000 birds and generates ~16.7 million liters of wastewater per week. Approximately every 10 days, ~950 L of PPW was withdrawn from the

Table 6.1: Soluble nutrient composition in poultry processing wastewater used in this study.

Parameters	Phase I (n = 6) [a]	Phase II (n = 6) [a]	Phase III (n = 3)	Phase IV (n = 5) [a]	Phase V (n = 5) [a]	Phase VI (n = 4) [a]
sCOD (mg L^{-1})	443.3 ± 89.5	608.3 ± 64.8	605.0 ± 189.0	730.2 ± 171.4	678.3 ± 161.4	546.5 ± 266.0
pH	7.1 ± 0.1	7.1 ± 0.2	7.1 ± 0.1	6.9 ± 0.2	7.0 ± 0.3	7.1 ± 0.2
TN (mg L^{-1})	70.5 ± 5.1	89.0 ±. 23.5	104.7 ± 15.0	68.5 ± 7.6	65.4 ± 16.5	112.8 ± 28.1
COD/N ratio	6.3 ± 1.4	7.1 ± 1.5	5.7 ± 1.0	10.6 ± 1.5	10.6 ± 2.2	4.7 ± 1.2
NH_4-N (mg L^{-1})	9.7 ± 5.9	9.8 ± 3.0	13.1 ± 2.9	11.4 ± 3.6	13.9 ± 7.5	6.9 ± 2.1
NO_2-N (mg L^{-1})	0.9 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NO_3-N (mg L^{-1})	0.2 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.3 ± 0.2	0.2 ± 0.1
PO_4-P (mg L^{-1})	11.9 ± 1.1	9.6 ± 4.7	13.2 ± 2.3	17.0 ± 5.7	10.2 ± 2.1	8.9 ± 3.4
SO_4-S (mg L^{-1})	7.8 ± 0.5	4.3 ± 2.7	2.5 ± 2.2	1.4 ± 0.3	6.3 ± 1.8	3.7 ± 0.7
K^+ (mg L^{-1})	48.8 ± 4.5	52.6 ± 7.2	61.9 ± 6.8	112.1 ± 82.4	55.8 ± 11.8	42.2 ± 13.8
Na^+ (mg L^{-1})	117.3 ± 11.8	110.6 ± 13.5	156.2 ± 81.8	272.8 ± 61.8	117.9 ± 30.5	70.4 ± 33.5
Cl^- (mg L^{-1})	65.5 ± 6.4	76.8 ± 8.4	82.5 ± 12.3	100.5 ± 32.9	60.7 ± 19.7	36.4 ± 6.9

[a] Number of fresh batches of wastewater collected within each operational phase.

liquid effluent stream of the facility's on-site dissolved air flotation (DAF) unit, stored temporarily in a stainless-steel tote, and transported to the experimental site on the same day and pumped continuously into the bioreactors. Table **6.1** shows the average nutrient composition of PPW used over the experimental period.

6.2.3 Experimental design

In the first round of experiments, three bioreactors were arranged in series in each treatment train, resulting in a cumulative HRT of 72 hrs (24 hrs per bioreactor). A 24-hr HRT was established for each bioreactor due to the relatively high sCOD and nutrient loading in PPW compared to municipal sewage (240 – 290 mg sCOD L^{-1}) where the typical residence time is 8 hrs [29,71]. This reactor HRT is also consistent with past studies on poultryponics [100]. However, use of shorter HRTs can lower treatment cost and so a second round of experiments was conducted with a total HRT of 48 hrs (16 hrs per reactor). In each configuration, both the algal and the bacteria-only bioreactor treatment trains were operated (Figure **6.1**). Although the series configuration eliminated bioreactor-level replication, each experiment was repeated three times to obtain system level replication.

The experiment lasted 310 days and comprised six operational phases. The treatment trains remained operational during short transition periods between phases to maintain microbial stability and system continuity. Phase I (Days 0–71) involved operation at 72-hr HRT to establish the baseline conditions. This was followed by three consecutive runs at 48-hr HRT (Phases II–IV; Days 72–142, 143–171, and 172–221, respectively) to assess the effects of reduced retention time. The system was switched back to 72-hr HRT for two additional experimental runs (Phases V and VI; Days 222–264 and 265–310, respectively). Each phase corresponded to a complete lettuce

production cycle, spanning seedling transplantation to harvest (Figure **6.1**). As noted previously, lettuce production methods and results are the subject of a separate publication.

6.2.4 Sampling and water quality analyses

Water samples were collected from the various sampling points in the treatment train three times per week, centrifuged (12,000 x g, 5 mins), and syringe filtered (0.22 μm Nylon). The resulting supernatant was stored at -80 °C for water quality analyses. Quantification of soluble inorganic ions was performed via suppressed ion chromatography on a Shimadzu Prominence Liquid Chromatography (LC) system (Shimadzu, Japan) [74]. Cations (Na^+, K^+, NH_4^+, Ca^{2+}, and Mg^{2+}) were measured using a Dionex IonPac CS16 column (4 × 250 mm, Thermo Scientific) and Dionex CERS 500e 4 mm regenerative suppressor. A Dionex IonPac AS22 column (4 × 250 mm, Thermo Scientific) plus regenerative suppressor (Dionex AERS 500 carbonate 4 mm) was used for the analysis of anions (Cl^-, NO_2^-, NO_3^-, PO_4^{3-}, and SO_4^{2-}). Total soluble nitrogen (TN), and soluble chemical oxygen demand (sCOD) were measured with the HACH assay per the manufacturer's instructions. Ammonium chloride (100 mg L^{-1}), and glucose (1 g L^{-1}) were used as external validation standards for each HACH assay, respectively.

Volatile fatty acids (C_1 – C_3) were quantified using a Shimadzu Prominence High-Performance Liquid Chromatography (HPLC) equipped with a Bio-Rad Aminex HPX-87H column and a photodiode array detector set at 210 nm [225]. The column oven temperature was 60 °C and the mobile phase consisted of 5 mM sulfuric acid, at a flow rate of 0.6 mL min^{-1}.

Untargeted LC-MS/MS analysis was performed to identify potential electron donors (carbon sources) for denitrification. The LC-MS/MS was a Vanquish UHPLC (Thermo Fisher, USA) coupled with an orbitrap mass spectrometer (Orbitrap Exploris 120, Thermo) using

electrospray ionization (H-ESI) in both positive and negatives modes with Xcalibur software for data acquisition. A 1 µL injection of sample was made on a C18 column (ACQUITY UPLC® BEH C18, 1.7 µm, 2.1 × 50 mm, Waters) at 40 °C with a 200 µL min^{-1} flow rate of mobile phase solution A (99.9% water and 0.1% formic acid) and solution B (99.9% methanol and 0.1% formic acid). The MS scan range was set at 70 – 700 m/z with resolution of 120,000. The compound selection criterion followed a requirement that they achieved an mzCloud annotation with a match factor >90%.

6.2.5 DNA extraction and 16S and 18S ribosomal deoxyribonucleic acid (rDNA) amplicon sequencing

At the end of each operational phase, the bioreactors were thoroughly mixed, and ~100 mL of culture was collected, centrifuged at 4,696 × g for 10 minutes, and washed three times with deionized water to remove residual salts. The resulting pellet was freeze-dried to determine total suspended solids (TSS) and stored at –80 °C. The FastDNA Spin Kit (MP Biomedicals) was used for DNA extraction according to the manufacturer's instructions. The QuantiFlour dsDNA system (Promega) was used for quantifying the extracted DNA concentration.

Targeted amplicon sequencing of the 16S rRNA gene (primers 515F/806R) and the 18S rRNA gene (primers 1391F/EukBr) was conducted by MR DNA (Molecular Research LP, Texas, USA) using an Illumina MiSeq platform. A HotStarTaq Plus Mix Kit (Qiagen, USA) was used for PCR amplification with the following conditions: initial denaturation at 95 °C for 5 minutes; 30 – 35 cycles of 95 °C for 30 secs, 53 °C for 40 secs, and 72 °C for 1 min; and a final extension at 72 °C for 10 mins. PCR products were verified via 2% agarose gel electrophoresis and the sequence data was processed using the sequencing center's proprietary ribosomal and functional

gene analysis pipeline as previously described by Wang et al. [74]. The original FASTQ files from this study have been uploaded to NCBI's Sequence Read Archive (SRA) under the accessions SUB15313150 and SUB15313191 for 16S and 18S rRNA sequences respectively.

6.2.6 qPCR for quantification of genes involved in nitrification and denitrification

qPCR was performed on a qTower3 instrument (Analytic Jena) with SYBR detection to target bacterial ammonia monooxygenase gene (amoA), *Nitrospira*'s nitrite oxidoreductase gene (nxrB), and denitrifying genes: nitrite reductase (nirK and nirS) and nitrous-oxide reductase (nosZ). *Nitrospira*'s nitrite oxidoreductase gene was selected based on the sequencing results. A 20 μL volume containing 2 μL of 10X diluted DNA template and 18 μL of PerfeCTa SYBR Green FastMix (QuantaBio) was used in all qPCR reactions with primer concentrations of 2.5 μM. The thermocycling conditions for each target gene are summarized in Appendix, Table A16. Dilutions of previously amplified product (cleaned using Qiagen's PCR purification kit and quantified using Promega's QuantiFluor dsDNA system) were used as standard [46]. Molecular grade dH_2O was used as a negative control and all measurements were performed in duplicate. The qPCR data were normalized to gene copy numbers per mL of reactor volume.

6.2.7 Nitrogen mass balance in bioreactors

The nitrogen mass balance was conducted to evaluate the impact of retention time on nitrogen dynamics in the bioreactors. The bioreactors were modeled under semi-steady-state conditions to evaluate the dynamics of NH_4-N, NO_2-N, NO_3-N, and organic nitrogen in treated effluents, averaged over daily intervals to reflect bioreactor performance [174]. The total mass of inorganic nitrogen from the bioreactors ($N_{inorg,eff,n}$) was calculated as the sum of soluble nitrogen

species in the effluent (Equation 6). Nitrogen loss (N_{lost}) was then determined by subtracting the total inorganic nitrogen, organic nitrogen, and nitrogen incorporated into microbial biomass from the TN input in PPW (Equation 7).

$$N_{inorg,eff,n} = \sum_{t=0}^{t=T} C_{n,t} \cdot Q_t \cdot \Delta t \quad \text{Equation 6}$$

$$N_{loss} = N_{influent} - N_{n,outflow} - N_{sludge} - orgN \quad \text{Equation 7}$$

where $N_{inorg,eff,n}$ is the mass of N in effluent, $C_{n,t}$ is the of inorganic nitrogen species (NH_4-N, NO_2-N, and NO_3-N) in the bioreactors over time *t*, and Q_t is the volumetric flow rate of bioreactor effluents. Nitrogen mass balances were averaged across the three replicated runs at each HRT. Pie charts were used to visualize the distribution of nitrogen across effluent species, microbial biomass, and losses for each bioreactor configuration.

6.2.8 Statistical analyses

Statistical analyses of 16S rRNA and 18S rRNA datasets were performed using R software (v4.4.0). Alpha diversity metrics (richness, Shannon, and Simpson indices) were calculated using the 'vegan' package [185]. Temporal shifts in community composition were assessed via non-metric multidimensional scaling (NMDS) using the 'phyloseq' package. Functional contributions of genes were predicted using Tax4Fun2 [226]. Descriptive statistics, independent t-tests, and graphical summaries were generated in Microsoft Excel. One-way ANOVA and post-hoc Tukey's HSD tests were conducted in R using the 'agricolae' and 'car' packages. Data that violated homogeneity of variance were log transformed before Tukey's multiple comparison test.

6.3 Results and discussion

6.3.1 Impact of hydraulic retention time on organics removal in bioreactors

PPW used in this study exhibited a high average soluble chemical oxygen demand of 598.4 ± 173.3 mg L^{-1} (Table **6.1**), with approximately 49% attributed to volatile fatty acids, primarily acetate, propionate and butyrate (291.9 ± 175.1 mg L^{-1}; Table **6.2**). These results are below those reported by Chen et al. [10], who observed an average of 1.1 g L^{-1} VFAs (C_1 to C_7) representing 97% of total COD in PPW. Such differences likely reflect variations in slaughterhouse operations, processing capacity, and pre-treatment approaches [13]. The sCOD in PPW promotes heterotrophic conditions and robust ammonification, consistent with previous findings [100]. This mineralization process increases pH (pH = 7 – 9; Figure 6.2A), necessitating effective pH control strategies to avoid adverse effects on downstream plant growth [100,174].

Under the 72-hr HRT operation (Phases I, V, and VI), both algal and bacterial bioreactors demonstrated similar sCOD removal efficiencies except for Phase V in which case the algal bioreactors had higher sCOD in the effluent (126 mg L^{-1}) than the bacterial bioreactors (84 mg L^{-1}; Table **6.3**). Effluent sCOD removal across the 72-hr HRT phases ranged from 80.3 – 95.9% for the algal bioreactors and 86.6 – 95.6% for the bacterial bioreactors (Table **6.3**). VFAs (acetate, propionate and butyrate) were not detected in the effluents during Phase I, suggesting active heterotrophic degradation (Table **6.2**). DO profiles supported this trend, with the lowest DO levels recorded in the first bioreactors (R1) of each train (Figure 6.2B). The algal bioreactors supported significantly higher DO levels than the bacterial bioreactors, given the role of photosynthesis (Figure 6.2B). ORP was also nominally higher in the algal bioreactors, but it was only significantly lower in algal R1 (Figure 6.2C).

Table 6.2: Volatile fatty acids in poultry processing wastewater, treated effluents and removal efficiencies (%) in bioreactors.

Phase (HRT)	Total volatile fatty acids (acetate, propionate, and butyrate)				
	Influent concentration (mg L^{-1})	Algal bioreactors		Bacterial bioreactors	
		Effluent concentration (mg L^{-1})	Removal efficiency (%)	Effluent concentration (mg L^{-1})	Removal efficiency (%)
I (72hrs HRT)	136.0 ± 31.5	nd	-	nd	-
II (48hrs HRT)	186.0 ± 31.1	40.7 ± 29.5 [a]	78.2 ± 14.5 [b]	13.3 ± 5.9 [b]	92.4 ± 4.8 [a]
III (48hrs HRT)	234.8 ± 83.3	25.5 ± 22.3 [a]	86.5 ± 17.4 [a]	45.0 ± 36.8 [a]	74.6 ± 28.0 [a]
IV (48hrs HRT)	316.9 ± 76.1	5.4 ± 2.3 [b]	99.0 ± 2.2 [a]	26.4 ± 21.5 [a]	92.4 ± 10.9 [b]
V (72hrs HRT)	524.4 ± 143.6	42.8 ± 32.6 [a]	92.3 ± 8.7 [a]	61.2 ± 54.1 [a]	87.8 ± 14.6 [a]
VI (72hrs HRT)	405.8 ± 245.3	nd	-	34.7 ± 25.0 [b]	85.5 ± 14.8 [b]

Values are means ± standard deviation of the independent batches of PPW collected within each Phase. Means of effluent concentrations and removal efficiencies within the bioreactors with similar letters are not statistically different based on the Tukey's HSD test ($p>0.05$). nd: not detected; hence removal efficiency was not estimated.

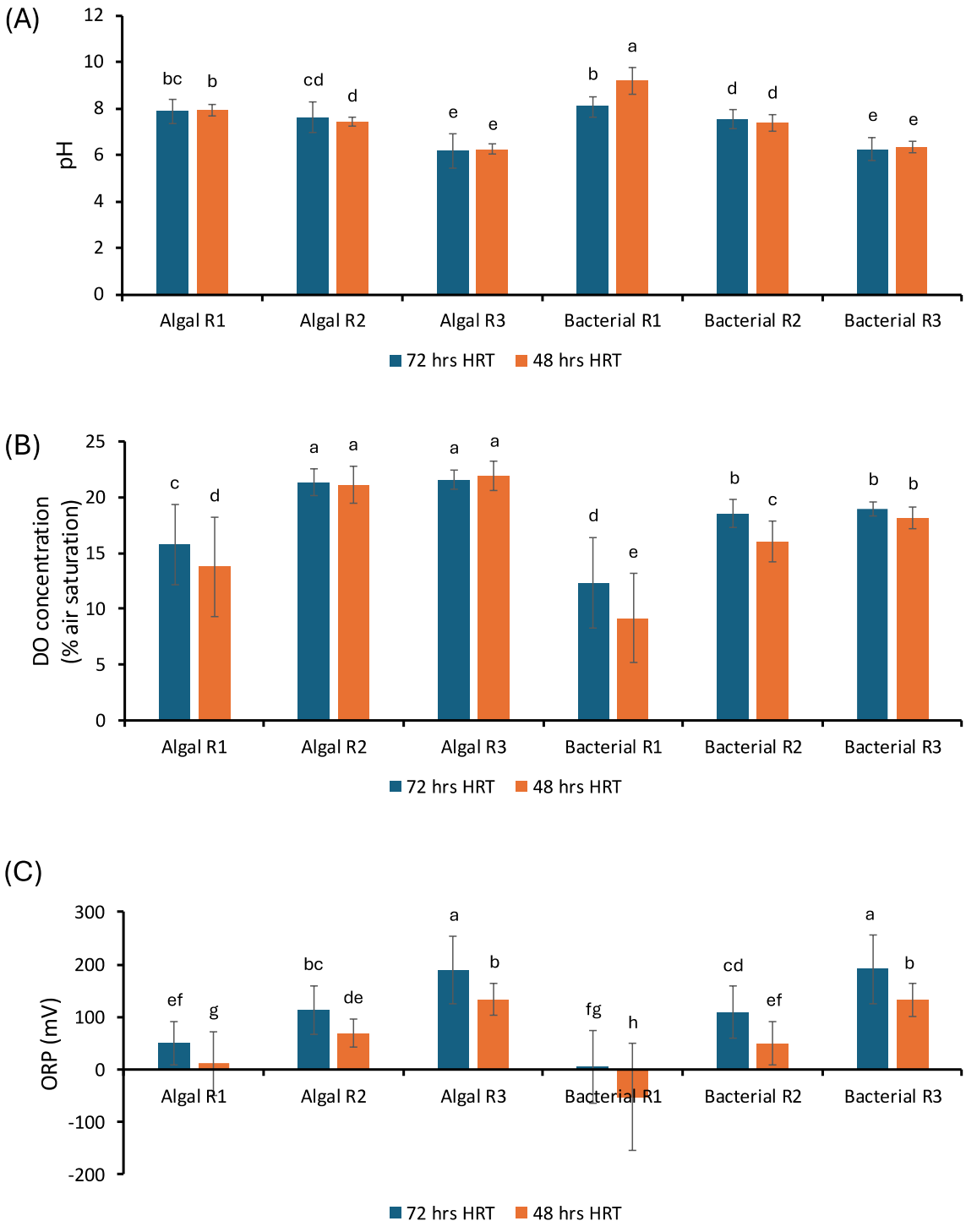


Figure 6.2: pH measurement (A), dissolved oxygen concentration (B) and oxidative-reductive potential (C) in bioreactors operating at 72hrs HRT and 42hrs HRT. Values are means ± standard deviation of 3 biological replicates. Within each bioreactor, means with similar letters are not statistically different based on the Tukey's HSD test ($p>0.05$).

The visible presence of green algae in the algal train, VFA consumption, and DO levels below saturation in R1 suggests the bioreactor was operating in mixotrophic/heterotrophic mode, similar to a bacterial system [82,227] when compared to algal R2 and R3 (Figure 6.2B). After organics were degraded in bioreactor 1, DO and ORP both increased in the subsequent bioreactors, regardless of treatment train (Figure 6.2).

Transitioning to a 48-hr HRT (Phases II – IV) increased the organic loading rate (OLR) from 147.8 ± 29.8 mg sCOD L^{-1} d^{-1} to an average of 325.6 ± 70.5 mg sCOD L^{-1} d^{-1}, nearly a 2.2-fold increase (Table **6.3**). This shift impacted effluent quality, particularly in the algal bioreactors, which showed sCOD removal efficiencies of 82.6% to 92.8% (Table **6.3**). In contrast, the bacterial bioreactors maintained higher removal efficiency (90.4% – 96.5%), with average effluent sCOD of 10.8 – 66.4 mg L^{-1} (Table **6.3**). The higher OLR supported rapid heterotrophic growth (as MLSS; Table **6.4**) and oxygen demand. In both treatment trains, bioreactor 1 had lower DO levels compared to the 72-hr HRT phases (Figure 6.2B). As before, the algal bioreactors sustained higher DO across all three bioreactor compartments. This advantage in oxygen generation, however, did not translate to better sCOD removal (Table **6.3**). Algae are known to secrete organic molecules (photosynthate) into water and have been documented to elevate sCOD in wastewater in past research [74,227]. Possibly compounding this issue, there was substantial variation in influent VFAs to the treatment train across phases, reaching an average of 524.4 mg L^{-1} in Phase V (Table **6.2**). Although many algae can consume VFAs, butyric acid and propionic acid in particular become toxic to green algae around 450 mg L^{-1} [228]. These findings suggest that while algae supply DO to heterotrophic bacteria, their metabolic byproducts may hinder overall organic removal efficiency under shorter retention times and higher OLR conditions.

Table 6.3: Soluble COD in poultry processing wastewater, treated effluents and removal efficiencies (%) in bioreactors

Phase (HRT)	sCOD					
	Influent concentration (mg L^{-1})	Average organic loading rate (mg sCOD L^{-1} d^{-1})	Algal bioreactors		Bacterial bioreactors	
			Effluent concentration (mg L^{-1})	Removal efficiency (%)	Effluent concentration (mg L^{-1})	Removal efficiency (%)
I (72hrs HRT)	443.3 ± 89.5 [a]	147.8 ± 29.8 [c]	22.0 ± 20.0 [a]	95.9 ± 4.4 [a]	20.8 ± 17.2 [a]	95.6 ± 3.2 [a]
II (48hrs HRT)	608.3 ± 64.8 [a]	304.2 ± 32.4 [ab]	104.2 ± 25.1 [a]	82.6 ± 5.2 [b]	20.5 ± 13.8 [b]	96.5 ± 2.6 [a]
III (48hrs HRT)	605.0 ± 189.0 [a]	302.5 ± 94.5 [ab]	47.6 ± 40 [a]	92.8 ± 8.3 [a]	10.8 ± 7.7 [a]	98.7 ± 1.9 [a]
IV (48hrs HRT)	730.2 ± 171.4 [a]	365.1 ± 85.7 [a]	98.7 ± 42.0 [a]	85.7 ± 7.1 [b]	66.4 ± 15.6 [b]	90.4 ± 3.9 [a]
V (72hrs HRT)	678.3 ± 161.4 [a]	226.1 ± 53.8 [bc]	125.7 ± 42.3 [a]	80.3 ± 8.2 [b]	84.1 ± 18.6 [b]	86.6 ± 6.1 [a]
VI (72hrs HRT)	546.5 ± 266.0 [a]	182.2 ± 88.7 [bc]	62.5 ± 7.7 [a]	87.1 ± 4.0 [a]	62.4 ± 14.1 [a]	87.2 ± 5.1 [a]

Values are means ± standard deviation of the independent batches of PPW collected within each Phase. Means of effluent concentrations and removal efficiencies within the bioreactors with similar letters are not statistically different between treatment based on the Tukey's HSD test ($p>0.05$).

Table 6.4: Summary of biomass concentration in bioreactors.

Phase	Parameters	Algal bioreactor 1	Algal bioreactor 2	Algal bioreactor 3	Bacterial bioreactor 1	Bacterial bioreactor 2	Bacterial bioreactor 3
72-hr HRT	MLSS (g L^{-1})	0.59	0.45	1.22	0.49	0.55	0.22
(Day 71)	SRT (days)	3.15	1.20	19.27	18.73	6.99	13.79
48-hr HRT	MLSS (g L^{-1})	0.84	0.51	0.95	1.26	0.45	0.48
(Day 142)	SRT (days)	4.98	3.51	21.34	48.62	6.19	17.68
48-hr HRT	MLSS (g L^{-1})	2.16	0.98	0.89	1.30	2.64	0.28
(Day 221)	SRT (days)	74.14	5.80	39.24	10.98	64.32	25.01
72-hr HRT	MLSS (g L^{-1})	1.85	0.73	1.72	0.93	0.66	0.67
(Day 264)	SRT (days)	8.15	1.41	57.25	6.21	7.04	26.93

MLSS: Mixed liquor suspended solids
SRT: Solids retention time

6.3.2 Nitrification response to substrate loading conditions in bioreactors

There was strong ammonification in the anaerobic wastewater holding tank prior to bioreactor treatment. This was indicated by high soluble NH_4-N from mineralization of organic compounds such as proteins, amino acids, and fats in PPW [10,213], which accounted for ~87% of TN (Table **6.1** & Figure **6.3**). The transformation of org-N into soluble ammonium is beneficial in Poultryponics, as ammonium can be readily converted to nitrate for plant uptake. In previous research, there was no significant nitrification (<3.5 mg L^{-1} NO_3-N) using parallel bioreactors operated continuously at 24-hr HRT (>220 days) [100]. The purpose of this multistage bioreactor setup and extending the overall treatment time was to encourage near-complete nitrification in bioreactors. In this regard, both bioreactors (algal and bacterial) were generally effective at both HRTs. However, important differences emerged between the two system types.

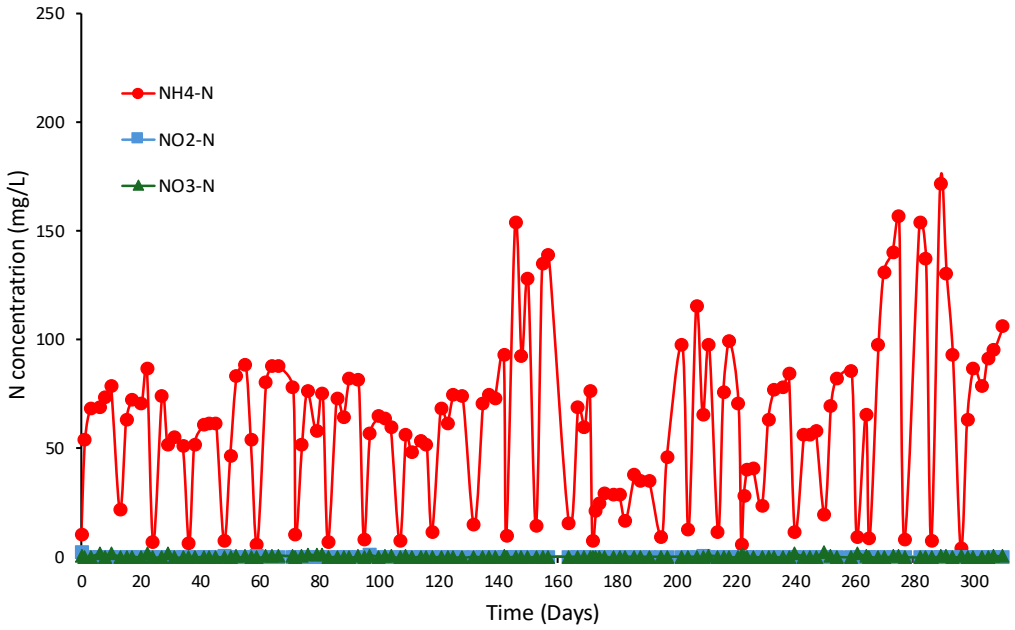


Figure 6.3: Time course plot of NH_4-N, NO_2-N and NO_3-N concentrations in PPW showing degradation of organic nitrogen to NH_4-N in the anaerobic wastewater holding tank.

Under 72-hr HRT, algal bioreactors 1 and 2 generally exhibited greater nitrification than the bacteria system (Figure **6.4** & Figure **6.5**). This was particularly evident during Phase I where algal R1 exhibited remarkable nitrification for 71 days (average NO_3-N concentration: 30.6 ± 15.6 mg L^{-1}, ~43% of TN; Figure **6.4**). High DO in algal R1 (~6.0 mg L^{-1}; Figure 6.2B) likely enhanced nitrification given that <3.5 mg L^{-1} was measured in the earlier study [66,100]. Additionally, differences in microbial communities in the inoculum between studies may have also contributed to variations in nitrifier activity. Although nitrification in algal R1 declined in Phases II – IV, nitrification proficiency re-emerged upon restoring the 72-hr HRT in Phases V and VI (Figure **6.4** & Figure **6.5**). In those last two phases, NO_2-N levels in R1 (18.6 ± 15.8 mg L^{-1}) and NO_3-N levels in R2 (44.8 ± 23.8 mg L^{-1}) of the algal system exceeded those of the corresponding bacterial bioreactors (7.1 ± 7.8 mg L^{-1} and 37.2 ± 17.4 mg L^{-1}, respectively), coinciding with elevated DO and ORP levels (Figure 6.6).

Moreover, the ability of algal R1 to simultaneously degrade organic matter and support nitrification suggests a functionally robust microbial community compared to bacterial R1 [46]. In contrast, bacterial R1 exhibited significantly lower ($p<0.05$) DO concentration (Figure 6.2B) and correspondingly lower nitrification (average NO_3-N concentration: 10.5 ± 14.2 mg L^{-1}, ~10% of TN; Figure **6.5**) with complete nitrification achieved downstream in bacterial R3 (Figure **6.5**). These results suggest that illumination and the presence of algae in algal bioreactors promoted early-stage nitrification by sustaining higher DO levels.

Bioreactors 3 in both systems ensured complete nitrification under 72-hr HRT operation. The sustained nitrification could be attributed to sludge recycling [229], CO_2 sparging, and high DO. CO_2 sparging, supports nitrification by supplying inorganic carbon to autotrophic nitrifiers [85,86,216], alleviating nitrite oxidation inhibition [71] and favors optimal pH for nitrification (Figure 6.2C).

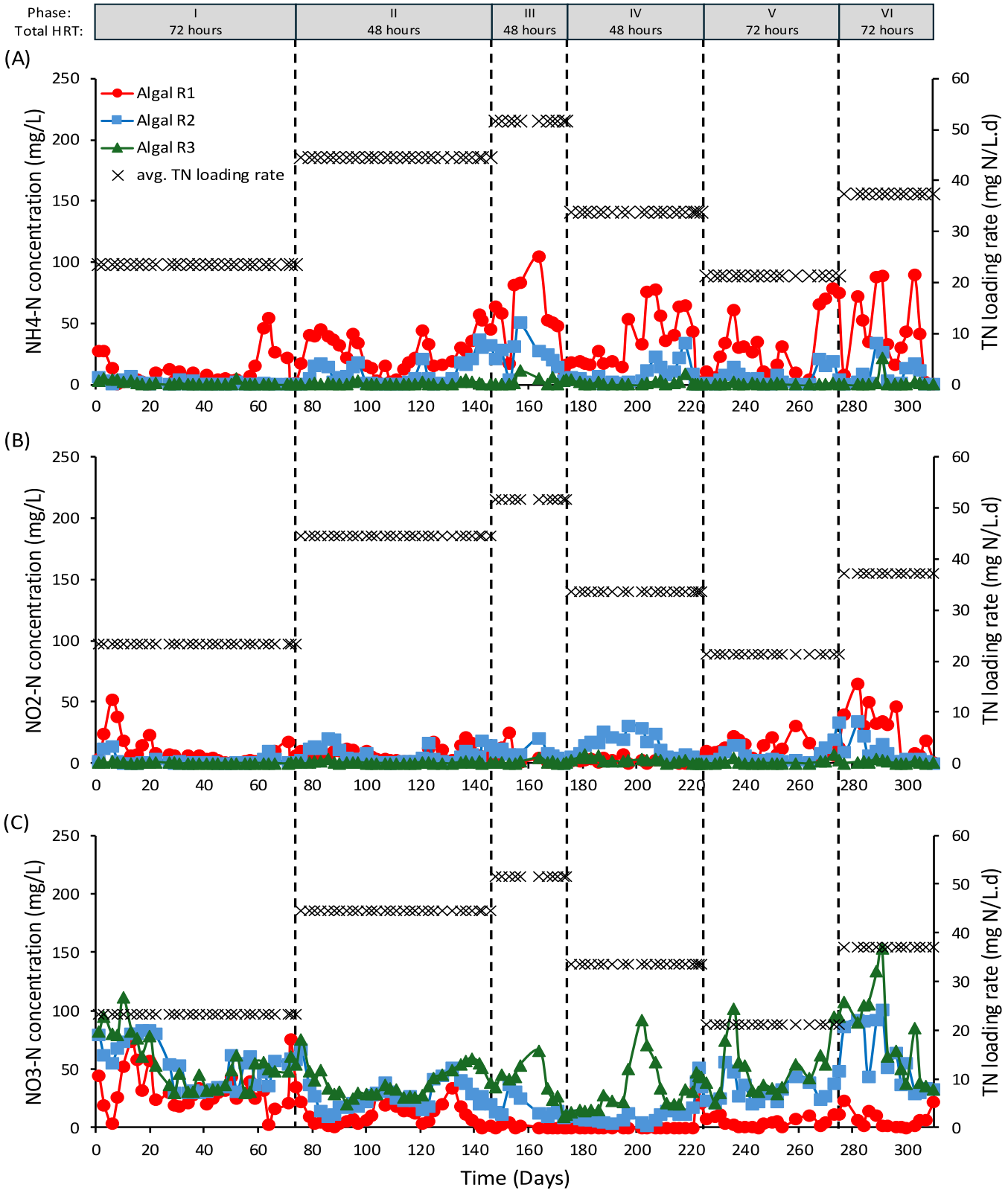


Figure 6.4: Time course plots of NH_4-N (A), NO_2-N (B), and NO_3-N (C) concentrations in effluents and TN loading rates of PPW in algal bioreactors 1, 2 and 3 across different hydraulic retention times.

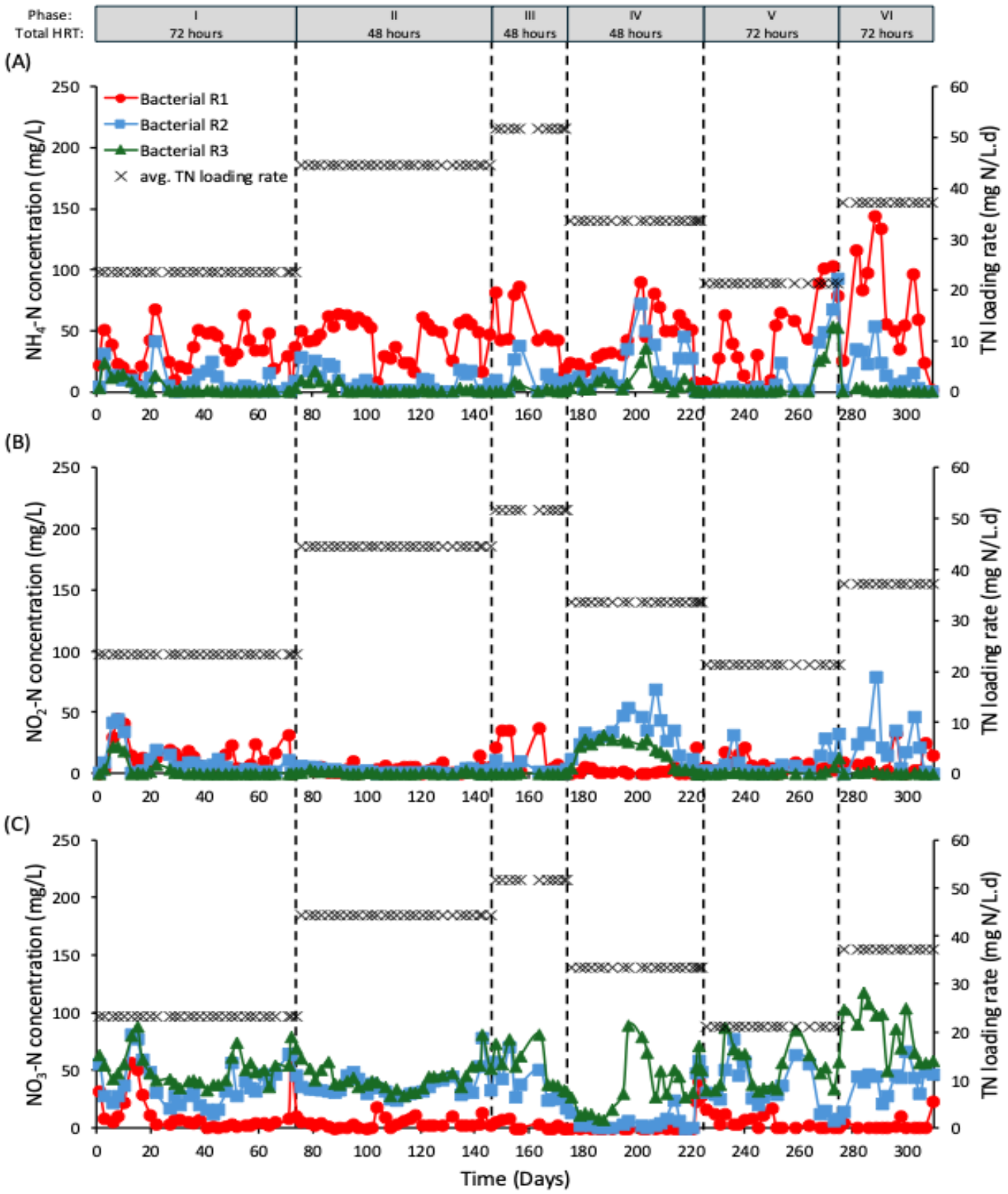


Figure 6.5: Time course plots of NH_4-N (A), NO_2-N (B), and NO_3-N (C) concentrations in effluents and TN loading rates of PPW in bacterial bioreactors 1, 2 and 3 across different hydraulic retention times.

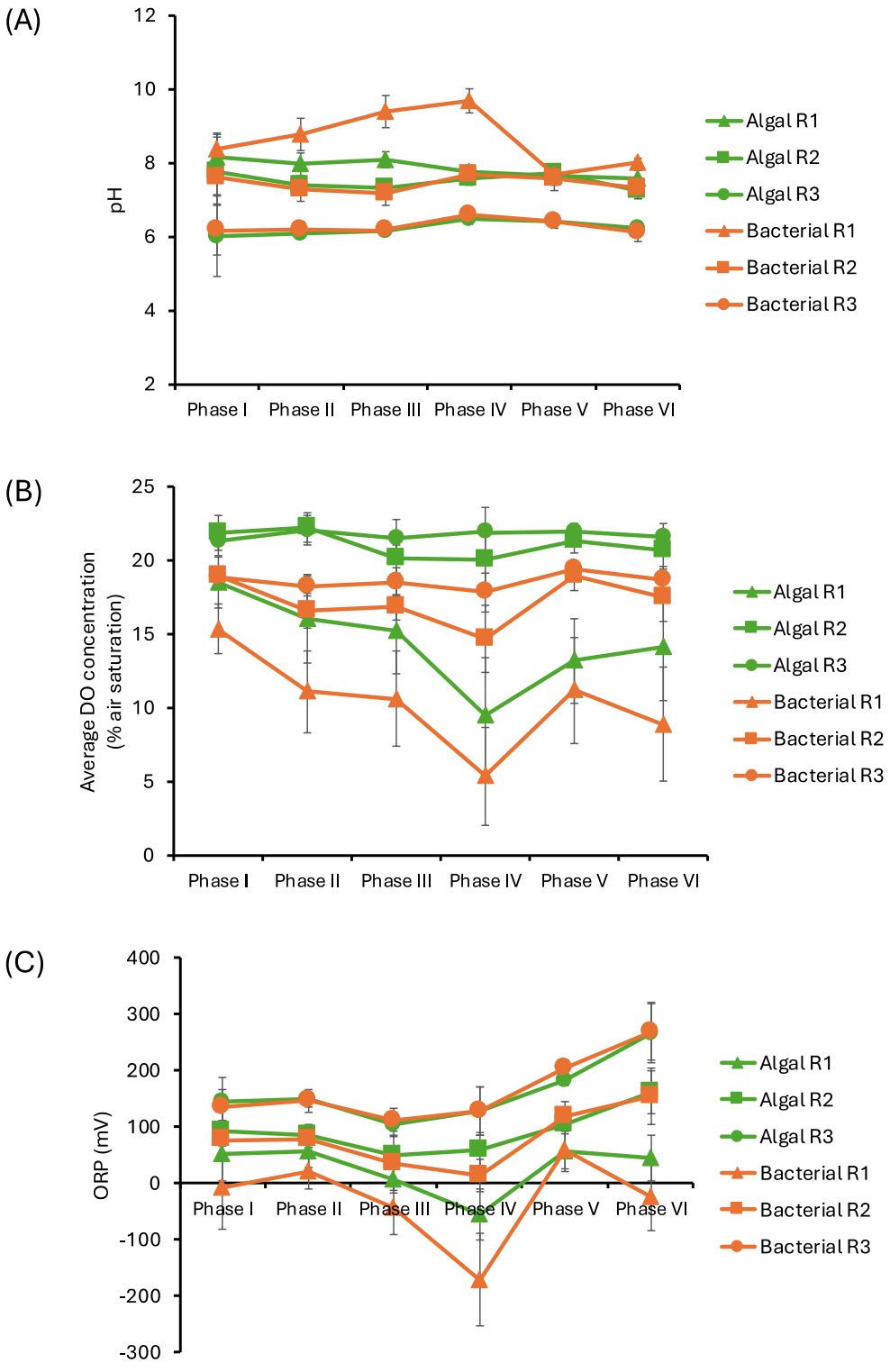


Figure 6.6: Average pH, DO and ORP in bioreactors over different operational phases. Values are means and standard deviations of multiple sampling times within each phase.

Switching to a 48-hr HRT in Phases II – IV resulted in a sustained decline in nitrification in both bioreactor types, culminating in a process upset in phase IV (Figure **6.4** & Figure **6.5**). In algal R1, average NO_3-N concentrations decreased from 14.7 ± 14.8 mg L^{-1} in Phase II to 0.0 ± 0.1 mg L^{-1} in Phase IV (Figure **6.4**). A similar trend was observed in bacterial R1, where the average NO_3-N concentration reduced from 6.2 ± 11.8 mg L^{-1} to 0.0 ± 0.1 mg L^{-1} within the same period. Between days 180 – 220 (Phase IV) there was a sudden inhibition of nitrite oxidation in bacterial R2 and R3, resulting in elevated NO_2-N concentrations (16.7 ± 11.3 mg L^{-1}; Figure **6.5**) in the treated effluents irrigating the hydroponics reservoirs (Figure **6.7**). Similarly, NO_2-N concentrations in algal R2 averaged 13.3 ± 9.5 mg L^{-1} (Figure **6.4**). However, algal R3 sustained nitrite oxidation with average NO_2-N levels in its effluent being only 2.5 ± 1.9 mg L^{-1} (Figure **6.4**), indicating higher NOB activity compared to bacterial R3 (Figure **6.11**).

The ORP in the bioreactors supports the observation that operating at a 48-hr HRT worsened nitrification (Figure 6.2C). Average ORP in all bioreactors across both trains had reduced significantly ($p<0.05$) compared to when they operated at 72-hr HRT (Figure 6.2C). The reduction in ORP, along with the loss in nitrifying capacity, coincided with increased nitrogen loading rates (NLR); from 23.4 mg N L^{-1} d^{-1} in Phase I to 44.5, 51.6, and 33.0 mg N L^{-1} d^{-1} in Phases II, III, and IV, respectively (Figure **6.4** & Figure **6.5**). These variations were partially driven by differences in influent TN concentrations (Table **6.1**) but mostly by the shorter HRT. Although elevated NLR is known to increase nutrient saturation and oxygen demand [230], it does not fully explain the sharp decline in nitrification observed in Phase IV, which had the lowest NLR (33.0 mg N L^{-1} d^{-1}) among the 48-hr HRT phases (Figure **6.4** & Figure **6.5**).

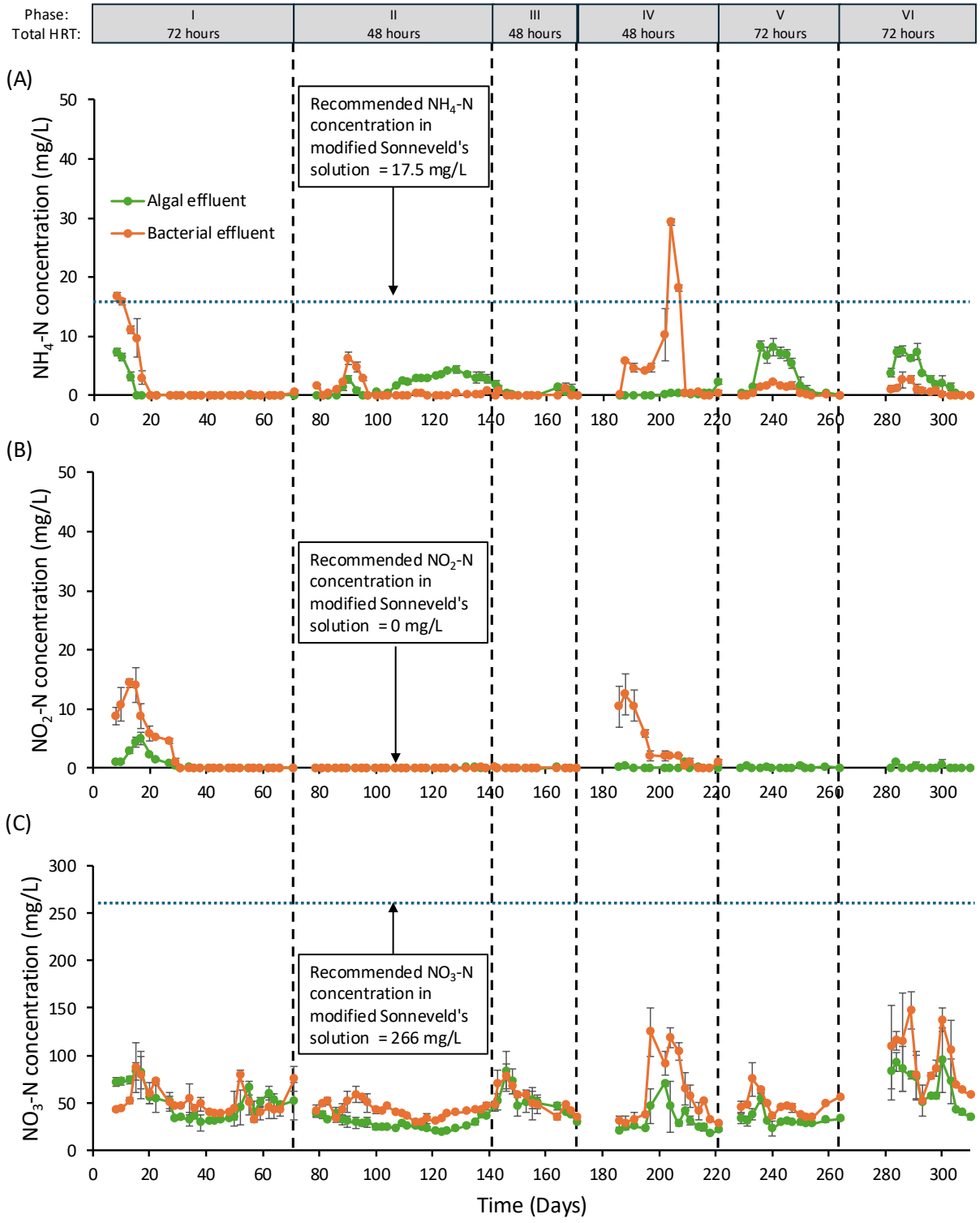


Figure 6.7: Time course plots of NH_4-N (A), NO_2-N (B), and NO_3-N (C) concentrations in grow beds. The broken blue line indicates the recommended threshold of NH_4-N, NO_2-N, and NO_3-N in hydroponic solution for lettuce cultivation (Sonneveld 1994) [73]. Error bars are standard deviations of 3 biological replicates.

During Phase IV, organic loading rate (OLR) in the bioreactors peaked at 365.1 ± 85.7 mg sCOD L^{-1} d^{-1}, reflecting the highest influent organic content (730.2 ± 171.4 mg L^{-1} sCOD, Table **6.3**). This spike in organic content increased the COD/N ratio to a maximum of 10.6 ± 1.5 (Table **6.1**) and reduced the average DO concentrations by 39%, 53% and 18% in algal R1, bacterial R1 and R2 respectively (Figure 6.6). Elevated COD/N ratios have been widely linked to NOB suppression and nitrite accumulation [78,231]. For example, Xu et al. reported ~94% nitrite accumulation at a COD/N ratio of 4 in a sequencing batch reactor treating domestic wastewater [78], while Peng et al. showed that a COD/N ratio of 4 increases the specific oxygen uptake rate of heterotrophic bacteria to 72% of total bacteria [232], limiting oxygen availability for nitrifiers under high OLR conditions. AOA and AOB have a higher oxygen affinity (half saturation constant, $K_{m,a}$ = 2.01 – 17.04 μM) than NOB ($K_{m,a}$ = 5.31 – 165.63 μM) [67] and could have contributed to the NO_2-N spike in Phase IV. Despite the moderate NLR (33.0 mg N L^{-1} d^{-1}) in Phase IV, the elevated OLR (365.1 ± 85.7 mg sCOD L^{-1} d^{-1}) and high COD/N ratio (10.6 ± 1.5) driven by shorter HRT likely intensified competition for oxygen, favoring heterotrophic metabolism and suppressing NOB activity. It is possible that algal DO production muted this upset as algal R2 and R3 exhibited smaller NH_4-N and NO_2-N spikes during Phase IV.

6.3.3 Nitrogen mass balance and chemical profiling to elucidate nitrogen losses

During the 48-hr HRT experiments (Phase II – IV), it became apparent that significant nitrogen loss was occurring in the bioreactors of both treatment trains, prompting a nitrogen mass balance to evaluate the impact of bioreactor retention time on nitrogen dynamics (Figure **6.8**).

At the 72-hr HRT, NO_3-N was the predominant soluble inorganic N in the reactor effluents, accounting for ~72% of TN (Figure **6.8**A & B). Biomass growth was notably higher in the algal

bioreactors (Table **6.4**), incorporating 24.2% of TN into microbial biomass, compared to 3.4% in the bacterial bioreactors (Figure **6.8**A & B). Nitrogen loss, defined as unaccounted TN, were minimal in the algal system (2.1%) but substantially higher in the bacterial system (19.3%). Given that the average pH in R1 were 7.9 ± 0.5 and 8.1 ± 0.4 in the algal and bacterial systems respectively (Figure 6.2A), denitrification appeared to be the main mechanism of nitrogen loss. Especially in the bacterial bioreactors, the average ORP in R1 (5.8 ± 69.0 mV) was within the typical range for denitrification (-50 and +50mV) [233]. In contrast, ORP in algal R1 was substantially higher (50.2 ± 41.4mV), suggesting a lower potential for denitrification at the 72-hr HRT (Figure 6.2).

Operating at 48-hr HRT increased nitrogen loss in both bioreactor systems, reaching 43.0% of TN in the algal bioreactors and 27.5% of TN in the bacterial bioreactors (Figure **6.8**C & D). The average pH in bacterial R1 during the 48-hr HRT operation was 9.2 ± 0.6 (Figure 6.2A), indicating potential ammonia volatilization [39]. In contrast, algal R1 maintained a lower pH (7.9 ± 0.3; Figure 6.2A), suggesting denitrification was the dominant nitrogen loss pathway. Extended operation (~150 days) at 48-hr HRT led to increased biomass accumulation (~2.2 g L^{-1} in algal R1 and ~1.3 g L^{-1} in bacterial R1; Table **6.4**), which likely created localized anoxic zones within biofilms [218]. These microenvironments support denitrifiers despite bulk-phase oxygenation, due to internal DO gradients limiting diffusion into dense biomass layers [218,234].

VFAs (acetate, propionate and butyrate) in PPW served as electron donors for denitrification [219–221]. VFA removal efficiency was significantly higher ($p<0.05$) in algal bioreactors than in bacterial ones during the 48-hr HRT, especially in Phase IV, where denitrification was very intense (Table **6.2**). Acetate, which has a high specific denitrification rate (0.091 NO_3-N $gCOD^{-1}$ d^{-1}) compared to propionate and butyrate [221,235], was removed at >94%

(Table **6.5**).In addition, significantly higher sCOD levels in the algal effluents (Table **6.3**) suggest that algal-derived metabolites or photosynthates could have contributed residual carbon that further accelerated heterotrophic denitrification in the algal bioreactors [236,237]. This is supported by the strong positive correlation between sCOD and nitrogen loss in the algal bioreactors while the bacterial system showed a nonlinear relationship (Figure **6.9**F).

In addition to VFAs, amino acids and organic acids in PPW may have supported N loss under high OLR conditions (Table **6.6**). Phenylalanine and tryptophan, following deamination in the anaerobic wastewater tank, contribute to VFAs and ammonium, with the former serving as denitrification substrate under high loading conditions [221].

Importantly, nitrogen is not a limiting nutrient for lettuce production in poultryponics [174]. Therefore, higher nitrogen loss in the algal bioreactors may be potentially beneficial by reducing excess soluble nitrogen requiring further treatment and lowering costs associated with external carbon sources for denitrification [39].

6.3.4 Microbial community analysis

Microbial communities responded dynamically to operational changes as evidenced by distinct temporal clustering in the NMDS plots (Figure **6.9**A & B). Alpha diversity metrics (genus level) revealed increasing bacterial diversity from bioreactor R1 to R3 in both treatment trains, indicating spatial community structuring likely driven by localized DO gradients. In contrast, eukaryote richness remained lower overall and showed minimal variation across treatments (Table **6.7** and Table **6.8**). Notably, switching from 72-hr to 48-hr HRT did not significantly alter bacterial diversity in the bacterial bioreactors, whereas all algal bioreactors exhibited a marked increase in diversity under 48-hr HRT.

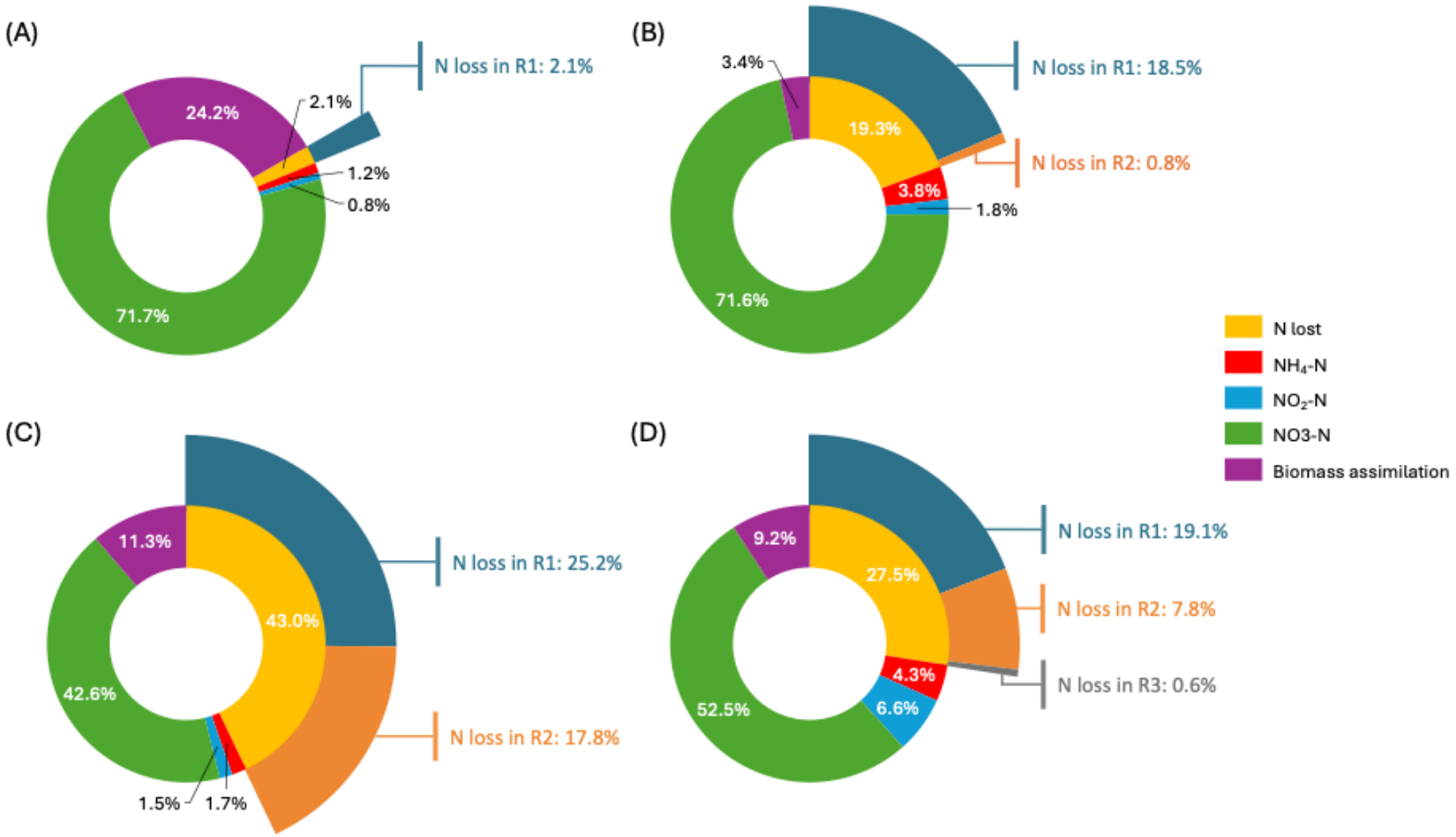


Figure 6.8: Nitrogen mass balance on bioreactors comprising of soluble nitrogen species (NH_4-N, NO_2-N and NO_3-N), nitrogen assimilation into microbial biomass and nitrogen lost via denitrification when operating at 72hrs HRT (A and B) and 42hrs HRT (C and D). Values are means of the 3 replicate experiments.

Table 6.5: Removal efficiency (%) of acetic acid acids in bioreactors.

Phase (HRT)	Algal bioreactors		Bacterial bioreactors	
	Effluent concentration (mg L^{-1})	Removal efficiency (%)	Effluent concentration (mg L^{-1})	Removal efficiency (%)
I (72hrs HRT)	nd	-	nd	-
II (48hrs HRT)	11.4 ± 8.2 [a]	94.0 ± 4.2 [b]	0.6 ± 1.1 [b]	99.7 ± 0.5 [a]
III (48hrs HRT)	nd	-	nd	-
IV (48hrs HRT)	nd	-	nd	-
V (72hrs HRT)	nd	-	nd	-
VI (72hrs HRT)	nd	-	nd	-

Values are means ± standard deviation of the independent batches of PPW collected within each Phase. Means of effluent concentrations and removal efficiencies within the bioreactors with similar letters are not statistically different based on the Tukey's HSD test ($p>0.05$).
nd: not detected; hence removal efficiency was not estimated

Table 6.6: Compounds in PPW that promote denitrification under higher loading rates in bioreactors

Compound	Match factor (mzcloud)	Relative peak area (PPW/hydroponic solution)	Role in denitrification
Suberic acid	98.9%	3.4	Direct oxidation by denitrifiers
Phenyllactic acid	99.5%	2103.7	Direct use by denitrifiers or formed during amino acid fermentation
Phenylalanine	99.5%	1092.9	Deaminated to VFAs, subsequently oxidized by denitrifiers
Tryptophan	92.1%	869.0	Deaminated to VFAs, subsequently oxidized by denitrifiers

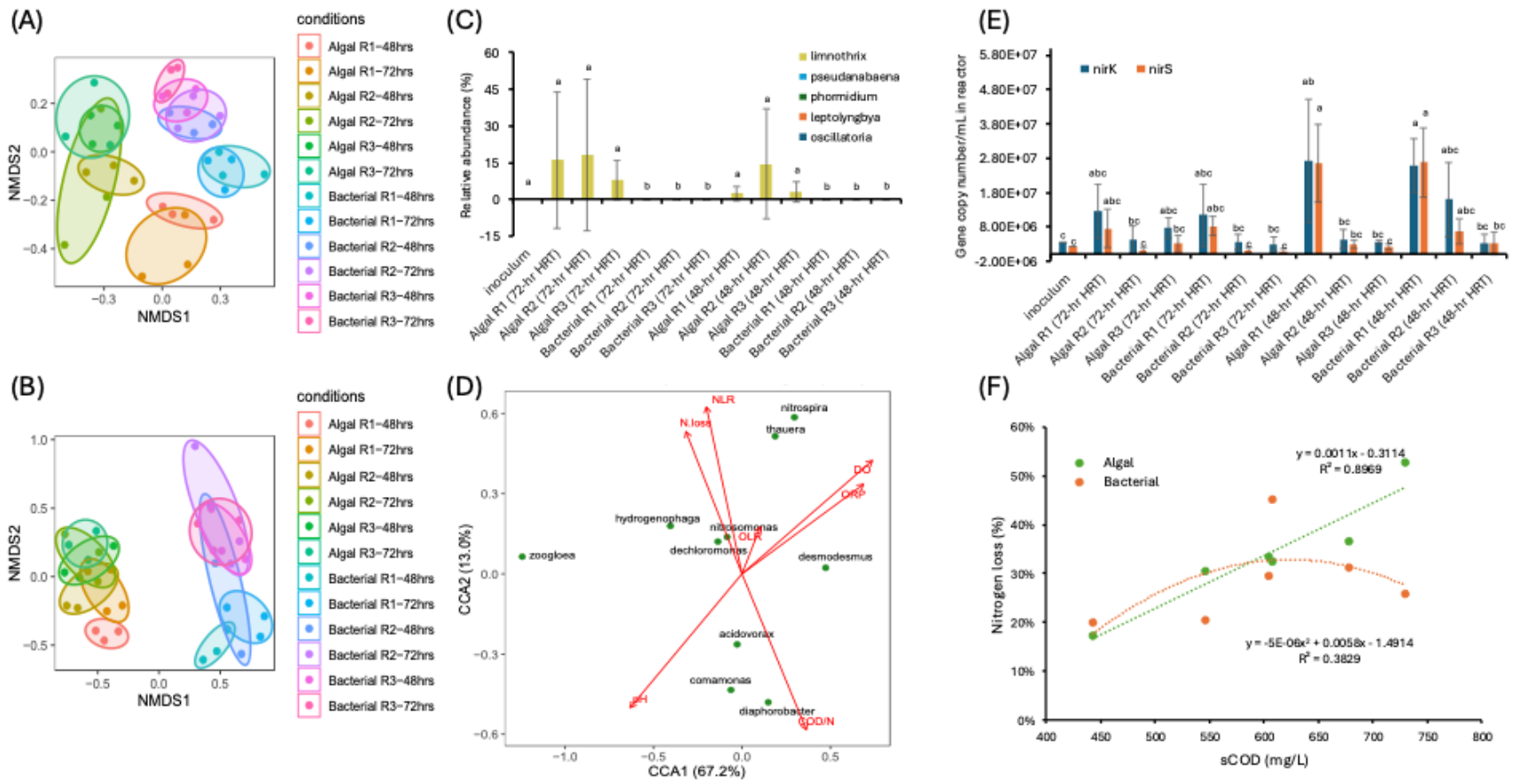


Figure 6.9: Non-metric multi-dimensional scaling (NMBS) plots of genus-level microbial community clustering based on 16S rRNA (A) and 18S rRNA genes in bioreactors (B). Relative abundance of cyanobacteria genera in bioreactors based on n=3 biological replicates (C). Relationship of dominant genera to nitrogen, sCOD, pH, DO, OLR and NLR (D). Denitrifying bacteria gene count concentration in bioreactors determined by quantitative PCR (E). Correlation between soluble COD (mg/L) and nitrogen loss (%) in bioreactors (F). Means with the same letters were not statistically significant and error bars are standard deviations of total abundances.

Pseudomonadota was the dominant bacterial phylum, comprising 47.6 – 79.7% of the microbial community across all bioreactors, followed by Bacteroidota at 6.9 – 44.0% relative abundance (Figure **6.10**A). Within Pseudomonadota, *Sphaerotilus*, commonly known as "sewage fungus" or "slime" [238], was prevalent under 72-hr HRT conditions, representing 2.0 – 32.7% of the microbial community.

Table 6.7: Alpha diversity indices at the genus level for prokaryote communities in bioreactors across operational phases.

	Alpha diversity indices		
	Richness	Shannon	Simpson
Inoculum	402.0	4.7	1.0
72-hr HRT (n=3)			
Algal R1	282.7 ± 10.4 ^{e}	2.9 ± 0.4 ^{de}	0.8 ± 0.1 ^{a}
Algal R2	322.3 ± 77.7 ^{cde}	2.4 ± 1.3 ^{e}	0.6 ± 0.3 ^{b}
Algal R3	390.0 ± 38.6 ^{ab}	4.1 ± 0.4 ^{ab}	0.9 ± 0.0 ^{a}
Bacterial R1	307.3 ± 18.1 ^{cde}	3.4 ± 0.1 ^{bcd}	0.9 ± 0.0 ^{a}
Bacterial R2	304.7 ± 23.6 ^{de}	2.9 ± 0.7 ^{de}	0.8 ± 0.1 ^{ab}
Bacterial R3	359.7 ± 11.9 ^{bc}	3.7 ± 0.1 ^{abcd}	0.9 ± 0.0 ^{a}
48-hr HRT (n=3)			
Algal R1	353.0 ± 12.5 ^{bcd}	3.9 ± 0.5 ^{abc}	0.9 ± 0.0 ^{a}
Algal R2	382.3 ± 27.3 ^{ab}	3.9 ± 0.4 ^{abc}	0.9 ± 0.1 ^{a}
Algal R3	426.0 ± 27.6 ^{a}	4.5 ± 0.1 ^{a}	1.0 ± 0.0 ^{a}
Bacterial R1	311.7 ± 11.0 ^{cde}	3.3 ± 0.2 ^{bcd}	0.9 ± 0.0 ^{a}
Bacterial R2	316.3 ± 32.0 ^{cde}	3.2 ± 0.2 ^{cde}	0.8 ± 0.0 ^{a}
Bacterial R3	349.0 ± 13.1 ^{bcd}	3.5 ± 0.4 ^{bcd}	0.9 ± 0.0 ^{a}

Values are means ± standard deviation of 3 biological replicates. Within each index, means with similar letters are not statistically different based on the Tukey's HSD test ($p>0.05$).

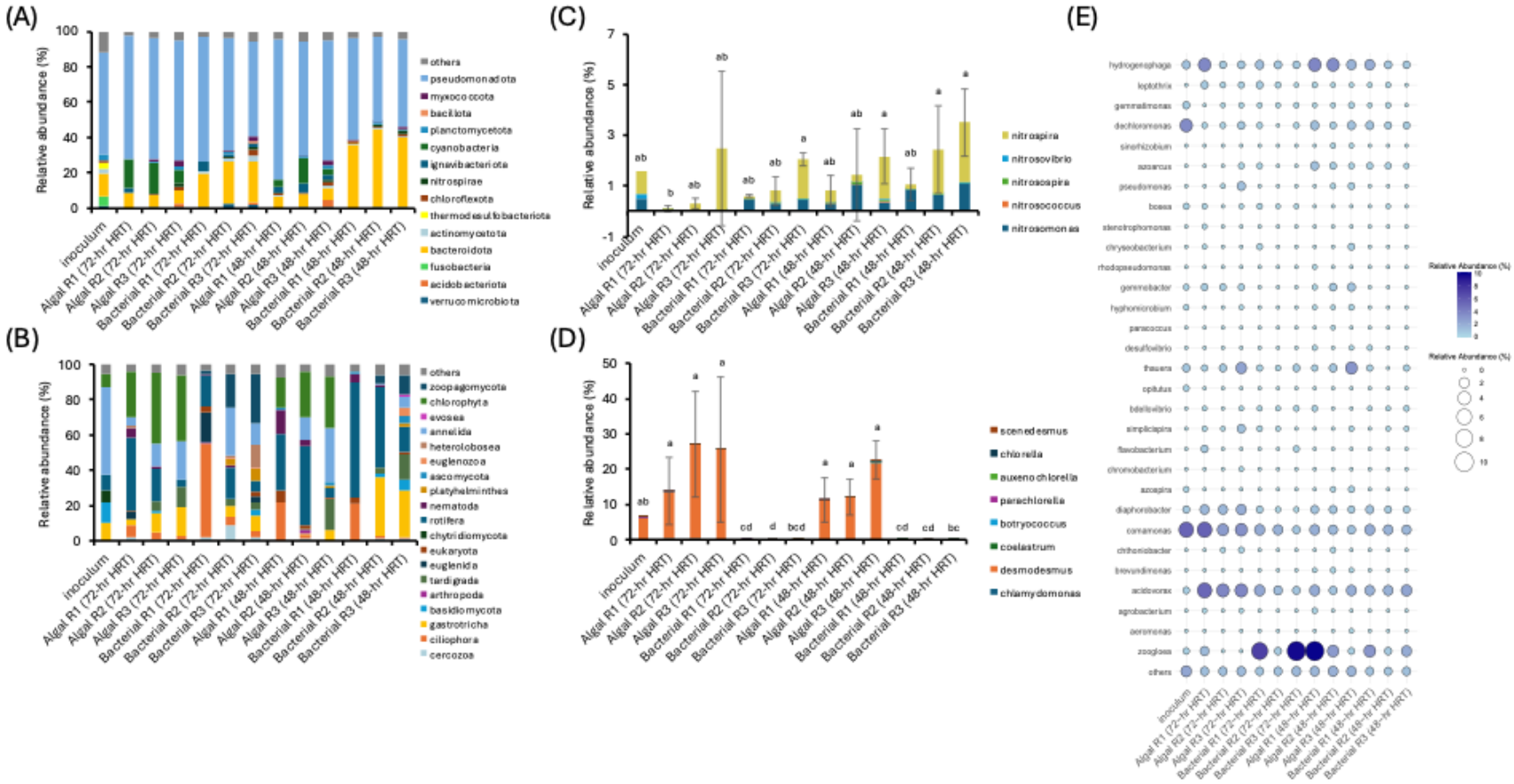


Figure 6.10: Relative abundance of phylum-level prokaryotes (A), phylum-level eukaryotes (B), nitrifying genera (C), green algae (D) and denitrifying genera (E) in bioreactors based on n=3 biological replicates. Taxa with relative abundance below 1% were assigned as others. Means with the same letters were not statistically significant and error bars are standard deviations of total abundances.

Table 6.8: Alpha diversity indices at the genus level for eukaryote communities in bioreactors across operational phases.

	Alpha diversity indices		
	Richness	Shannon	Simpson
Inoculum	108.0	2.0	0.7
72-hr HRT (n=3)			
Algal R1	76.0 ± 12.2 ^{b}	1.8 ± 0.8 ^{abc}	0.7 ± 0.2 ^{abc}
Algal R2	86.7 ± 30.3 ^{ab}	2.1 ± 0.5 ^{ab}	0.8 ± 0.1 ^{a}
Algal R3	89.7 ± 19.7 ^{ab}	1.9 ± 0.2 ^{abc}	0.8 ± 0.0 ^{ab}
Bacterial R1	61.7 ± 8.3 ^{b}	1.3 ± 0.6 ^{bc}	0.5 ± 0.2 ^{bcd}
Bacterial R2	64.3 ± 26.7 ^{b}	1.6 ± 0.3 ^{abc}	0.6 ± 0.1 ^{abcd}
Bacterial R3	91.0 ± 16.5 ^{ab}	1.9 ± 0.7 ^{abc}	0.7 ± 0.2 ^{ab}
48-hr HRT (n=3)			
Algal R1	78.0 ± 15.9 ^{b}	1.8 ± 0.3 ^{c}	0.7 ± 0.1 ^{abc}
Algal R2	82.3 ± 23.3 ^{ab}	1.9 ± 0.6 ^{c}	0.7 ± 0.1 ^{ab}
Algal R3	113.3 ± 20.6 ^{a}	2.2 ± 0.4 ^{a}	0.8 ± 0.1 ^{a}
Bacterial R1	63.7 ± 12.7 ^{b}	1.2 ± 0.3 ^{abc}	0.5 ± 0.1 ^{cd}
Bacterial R2	74.3 ± 26.7 ^{b}	1.1 ± 0.5 ^{abc}	0.5 ± 0.2 ^{d}
Bacterial R3	94.0 ± 16.5 ^{ab}	1.9 ± 0.3 ^{abc}	0.8 ± 0.1 ^{ab}

Values are means ± standard deviation of 3 biological replicates. Within each index, means with similar letters are not statistically different based on the Tukey's HSD test ($p>0.05$).

Sphaerotilus sp. are known to utilize a wide range of carbon substrates (organic acids, sugars, and alcohols), and are frequently detected in effluents from paper mills, sugar refineries, canneries, breweries, and municipal sewage [238,239]. However, its association with buoyant flocs and sludge bulking in activated sludge systems presents challenges for solids separation [240]. Other genera within Pseudomonadota, including *Neolewinella*, *Brachymonas*, *Zoogloea*, and *Rhodocyclus*, likely contributed synergistically to sCOD removal (>80%) by degrading complex organic substrates present in PPW (Appendix, Table A17).

Illumination of bioreactors promoted Chlorophyta (17.3% – 40.6% of eukaryotes) in the algal bioreactors (Figure **6**.**10**B). The most dominant green algae was *Desmodesmus* (11.1 – 26.6%), with minor contributions (<0.5%) from *Chlamydomonas*, *Coelastrum*, *Botryococcus*, *Parachlorella*, *Auxenochlorella*, and *Chlorella* (Figure **6**.**10**D). *Desmodesmus* is known to degrade oil-rich wastewaters under mixotrophic conditions [196,198], making it particularly suited for the high lipid content of DAF effluents [10]. Among prokaryotes, Cyanobacteria accounted for 3.2 – 18.1% of the community in algal bioreactors (Figure **6**.**10**A). *Limnothrix* was the most abundant cyanobacterium, while other genera such as *Oscillatoria*, *Leptolyngbya*, *Phormidium*, and *Pseudanabaena* were detected in low abundances (<0.1%; Figure **6**.**9**C). Although these cyanobacteria may contribute to nutrient removal, certain strains are known to produce cyanotoxins and off-flavor compounds, which could pose risks in downstream hydroponic applications [193,194].

The nitrifying genera detected in the bioreactors were composed primarily of canonical AOB, including *Nitrosomonas*, *Nitrosococcus*, *Nitrosospira*, and *Nitrosovibrio*, with *Nitrospira* as the dominant NOB (Figure **6**.**10**C). The abundance of genus *Nitrospira*, capable of complete ammonium oxidation to nitrate (comammox) [167] was consistently abundant in bioreactor R3 than in R1 in both treatment trains, likely due to elevated DO levels, continuous sludge recycling, and CO_2 sparging in the final-stage bioreactors (R3).

Under high OLR conditions in Phase IV (Day 221), *Nitrospira* abundance remained higher in the algal bioreactors (<1.7%) than in the bacterial system (<0.3%), coinciding with sustained nitrite oxidation in algal R3 and pronounced nitrite accumulation in bacterial R3 (Figure **6**.**4** & Figure **6**.**5**; Figure **6**.**11**). qPCR targeting the nitrite oxidoreductase gene (nxrB) specific to *Nitrospira* also showed that elevated COD/N ratios and OLR in Phase IV suppressed NOB activity

in the bacterial bioreactors. *Nitrospira* gene copies in algal R3 (7.33×10^6 mL^{-1}) were 68.5-fold higher than in bacterial R3 (1.07×10^5 mL^{-1}), supporting robust nitrite oxidation under high loading rates (Figure **6**.**11**C&D).

Furthermore, biotic interactions also contributed to nitrifier dynamics. *Desmodesmus*, the dominant green alga in this study, played a role in supporting *Nitrospira*. Canonical correspondence analysis (CCA) revealed that *Desmodesmus* was positively associated with DO and ORP, conditions favorable for nitrification (Figure **6**.**9**D). Although *Nitrospira* was not strongly colocalized with *Desmodesmus*, both occupied similar ordination region along CCA1, which explained 67.2% of the variance. These results align with previous findings that green algae enhance DO via photosynthesis [169,170] and alleviate inhibitors [46,47], thereby creating a favorable ecological niche for nitrifying bacteria under high organic loading.

On the contrary, there was a diverse assemblage of denitrifying genera in the bioreactors, including *Zoogloea*, *Comamonas*, *Acidovorax*, *Thauera*, *Hydrogenophaga*, *Dechloromonas*, and *Diaphorobacter* (Figure **6**.**10**E). During the 72-hr HRT, *Zoogloea* abundance peaked in bacterial R1 (7.2%) and R3 (9.7%), corresponding with the observed nitrogen loss of 19.3% in the bacterial train (Figure **6**.**8**B). However, under the 48-hr HRT, *Zoogloea* abundance in the algal bioreactors reached 9.9% in R1 and 2.8% in R2, mirroring the elevated nitrogen loss (43%; Figure **6**.**8**C). *Zoogloea* is an abundant denitrifying bacterium in activated sludge wastewater treatment plants, capable of utilizing VFAs, ethanol, and amino acids as electron donors [241,242]. Its production of extracellular polymeric substances (EPS) promotes floc formation and facilitate oxygen diffusion gradients that support denitrification in localized anoxic zones, as evidenced by negative correlation with DO and ORP (Figure **6**.**9**D).

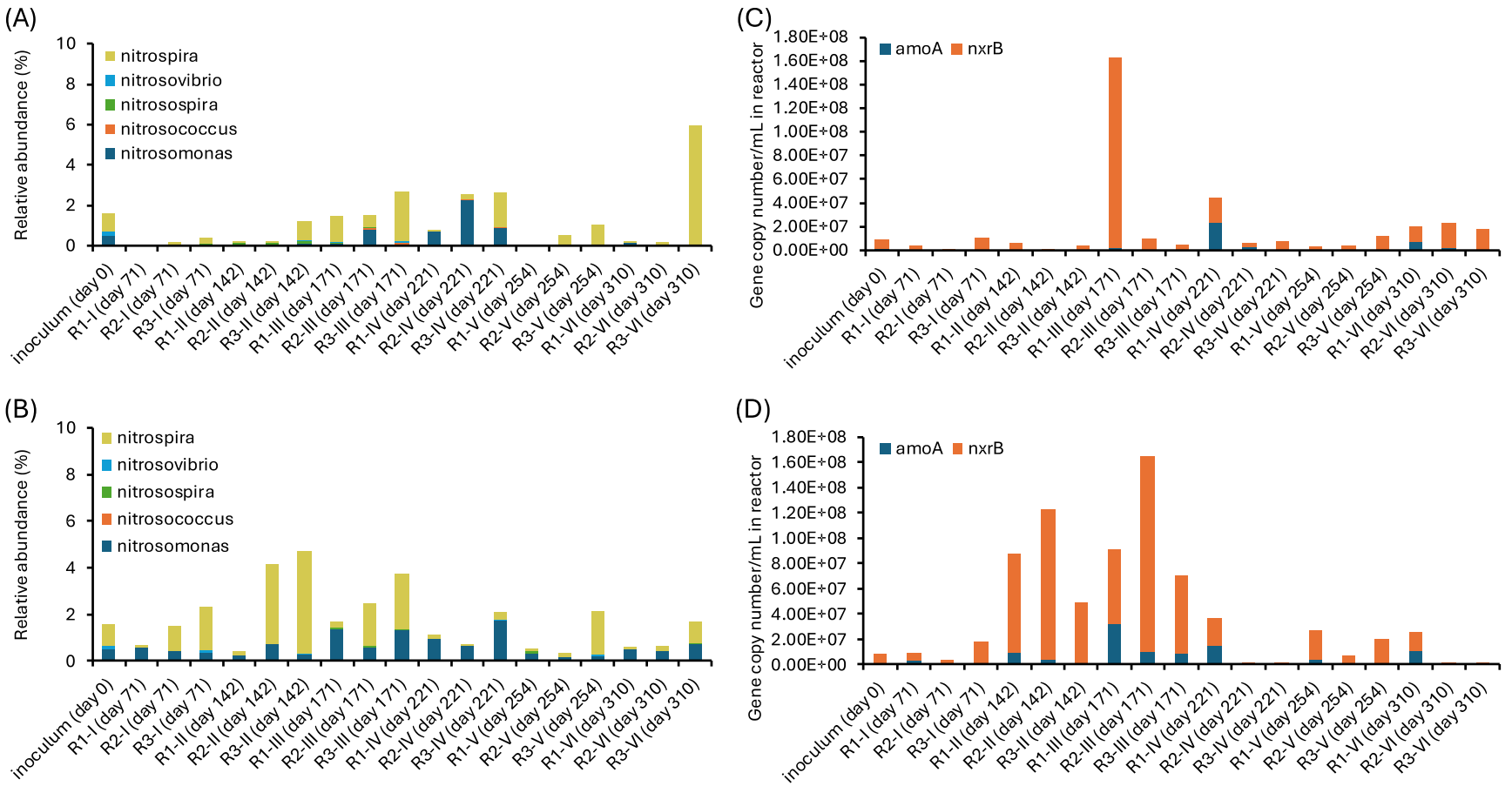


Figure 6.11: Time course relative abundance of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria genera in the algal bioreactors (A) and bacterial bioreactors (B). Time course gene count concentration of ammonia monooxygenase (amoA) and nitrite oxidoreductase (nxrB) in algal bioreactors (C) and bacterial bioreactors (D).

qPCR results corroborated this trend, with nitrite reductase gene copies (nirS and nirK) markedly higher at 48-hr HRT, especially in bioreactor R1, compared to 72-hr HRT (Figure **6**.**9**E). qPCR targeting nosZ failed to amplify in all samples, indicating either very low abundance or potential absence of nosZ-encoding populations. Functional gene prediction also revealed a 2.5 – 3.4-fold increase of nirK and nirS abundance over nosZ under the 48-hr HRT (Figure **6**.**12**), indicating an increased potential for incomplete denitrification and N_2O emissions (~310 times the global warming potential of CO_2) [243]. Together, these findings suggest that while nitrogen removal was enhanced under high organic loading, the risk of greenhouse gas emissions (particularly N_2O) from Poultryponics may also be elevated under these conditions.

6.3.5 Implications for hydroponic production

Successful upstream nitrification in the series bioreactors represents a significant improvement over previous parallel reactor operation, where high sCOD forced nitrification in the hydroponic reservoirs [100]. Thus, the current system offers flexibility for decoupled pH management specific to plant requirements (pH = 5 – 6), without compromising nitrogen transformation.

Despite significant N loss under 48-hr HRT and NO_3-N concentrations being approximately 84% lower than the 266 mg L^{-1} threshold in hydroponic lettuce solutions (Figure **6**.**7**), nitrogen could still be ruled out as a limiting nutrient for lettuce production [174]. However, the average concentrations of Na^+ (140 – 193 mg L^{-1}) and Cl^- (80 – 116 mg L^{-1}) in treated PPW, may induce salt stress [90], reducing lettuce biomass by disrupting osmotic regulation and interfering with the K^+ uptake [89], especially as average K^+ concentration ranged between 54 and 81 mg L^{-1} (Table **6**.**9**). High Na^+ levels in treated PPW, inhibits foliar K, Mg, Ca, Cu in hydroponic lettuce but can be mitigated by wholistic nutrient supplementation (N, P, K and micronutrients) [174]. The series

bioreactor operation, however, offers an opportunity for targeted nutrient supplementation (K and micronutrients) since N and P are not limiting nutrients in Poultryponics.

Despite the resilience of the Poultryponics in removing key pathogens such as *Salmonella*, *Escherichia coli*, and *Campylobacter* [100,204], the presence of other microbial taxa within the bioreactors raises food safety concerns. Notably, cyanobacteria, particularly *Limnothrix*, were consistently detected in the algal bioreactors (Figure **6.9**C). *Limnothrix* produces limnothrixin, a cyanotoxin with toxicological properties similar to β-N-methylamino-L-alanine (BMAA) [244,245], which may pose risks due to potential accumulation in edible plant tissues. These findings highlight the importance of monitoring and targeted management strategies to mitigate cyanobacterial proliferation, ensuring the safety of hydroponic lettuce irrigated with treated PPW.

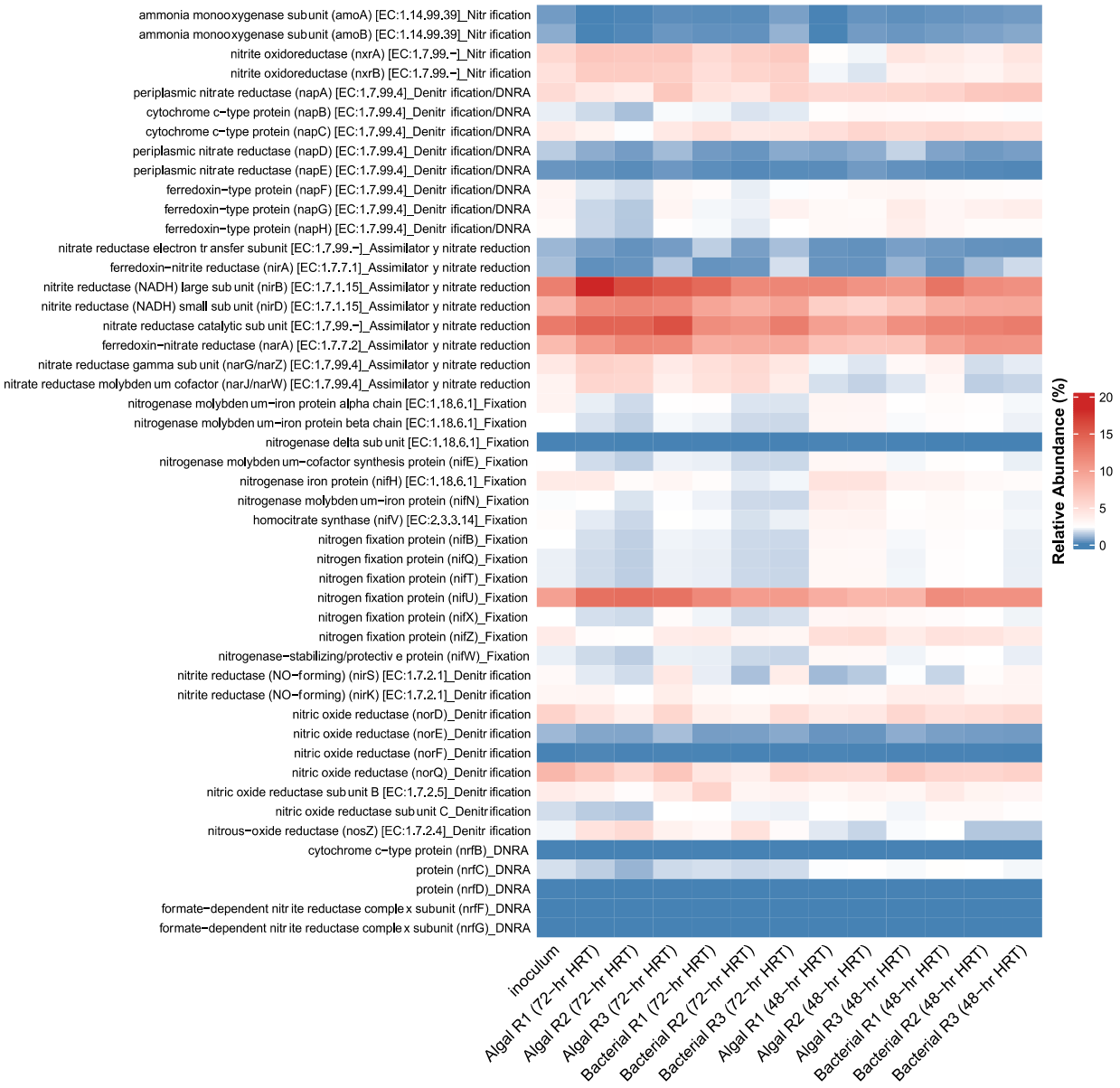


Figure 6.12: Heatmap representation of gene abundances for main bacterial N-cycle pathways.

Table 6.9: Nutrient concentration in treated effluents prior to hydroponic irrigation

Parameters	Phase I (72hrs HRT)	Phase II (48hrs HRT)	Phase III (48hrs HRT)	Phase IV (48hrs HRT)	Phase V (72hrs HRT)	Phase VI (72hrs HRT)
NH_4-N (mg L^{-1})	3.0 ± 1.9 (4.8 ± 3.9)	3.6 ± 1.3 (3.4 ± 1.9)	2.1 ± 1.8 (1.0 ± 0.5)	2.7 ± 2.4 (8.5 ± 7.4)	4.3 ± 2.5 (1.0 ± 0.8)	2.6 ± 2.5 (2.6 ± 2.6)
NO_2-N (mg L^{-1})	0.4 ± 0.4 (3.8 ± 1.5)	0.2 ± 0.1 (0.3 ± 0.2)	0.0 ± 0.0 (0.0 ± 0.0)	1.0 ± 1.0 (9.4 ± 8.3)	0.4 ± 0.3 (0.4 ± 0.2)	0.9 ± 0.8 (0.7 ± 0.3)
NO_3-N (mg L^{-1})	39.5 ± 15.9 (47.6 ± 14.2)	26.8 ± 8.9 (40.9 ± 8.4)	43.0 ± 15.4 (48.5 ± 13.3)	29.6 ± 21.2 (46.5 ± 39.1)	26.5 ± 6.3 (48.1 ± 25.0)	64.0 ± 25.7 (89.0 ± 39.5)
PO_4-P (mg L^{-1})	13.4 ± 5.0 (13.6 ± 3.7)	14.3 ± 5.5 (16.2 ± 8.7)	19.1 ± 7.0 (19.6 ± 7.9)	18.6 ± 12.2 (19.2 ± 11.6)	13.2 ± 1.6 (15.1 ± 5.5)	24.9 ± 11.1 (26.2 ± 12.9)
SO_4-S (mg L^{-1})	38.2 ± 23.8 (35.8 ± 21.1)	49.7 ± 18.2 (33.3 ± 15.7)	54.3 ± 26.5 (45.3 ± 18.7)	90.9 ± 30.0 (74.4 ± 20.2)	67.2 ± 23.0 (58.5 ± 26.8)	84.0 ± 42.4 (56.2 ± 32.3)
K^+ (mg L^{-1})	64.0 ± 15.4 (64.9 ± 16.6)	54.6 ± 6.4 (56.0 ± 8.8)	80.5 ± 24.4 (80.8 ± 23.1)	76.7 ± 28.4 (75.6 ± 28.2)	61.7 ± 8.1 (67.6 ± 24.5)	111.4 ± 47.1 (108.0 ± 54.4)
Na^+ (mg L^{-1})	158.4 ± 42.3 (163.7 ± 45.1)	139.8 ± 19.4 (140.9 ± 25.8)	183.7 ± 56.9 (180.8 ± 39.9)	192.8 ± 70.8 (186.8 ± 67.4)	160.6 ± 18.8 (173.4 ± 57.4)	188.5 ± 77.1 (187.4 ± 58.9)
Cl^- (mg L^{-1})	83.1 ± 20.4 (86.2 ± 21.7)	79.8 ± 9.7 (82.5 ± 11.2)	87.4 ± 25.0 (89.0 ± 22.4)	115.7 ± 71.0 (115.7 ± 68.2)	81.4 ± 12.2 (88.6 ± 33.4)	133.9 ± 58.4 (133.1 ± 62.7)
Electrical conductivity (uS cm^{-2})	0.88 ± 0.01 (0.89 ± 0.10)	0.96 ± 0.08 (0.95 ± 0.12)	0.91 ± 0.10 (0.90 ± 0.05)	0.99 ± 0.11 (0.98 ± 0.10)	0.95 ± 0.10 (0.92 ± 0.10)	1.08 ± 0.10 (1.06 ± 0.06)

Values are means ± standard deviation of the nutrient concentrations averaged over the experimental phase. Values in parenthesis represent those from the bacterial train while those without parenthesis are from the algal train.

6.4 Conclusion

This study evaluated the impact of series bioreactor configuration and retention time on organics removal and nitrogen dynamics in Poultryponics. At 72-hr HRT, both algal and bacterial treatment trains achieved high sCOD removal (80 – 96%) and sustained nitrification. Early-stage nitrification was predominant in the algal bioreactors due to higher DO (~6.0 mg L^{-1}) facilitated by *Desmodesmus* proliferation. Operating at 48-hr HRT increased organic and nitrogen loading rates by nearly two-fold, suppressing nitrite oxidation in bacterial bioreactors. Algal bioreactors maintained nitrification performance under these conditions, supported by higher *Nitrospira* abundance and favorable DO (~8.0 mg L^{-1}) and ORP (~135 mV). Dominant denitrifiers including *Zoogloea* and *Hydrogenophaga*, thrived under high COD/N (10.6) and OLR (365.1 ± 85.7 mg sCOD L^{-1} d^{-1}), utilizing VFAs (acetate, propionate and butyrate), amino acids, and algal metabolites as electron donors. While series bioreactor configuration improved upstream nitrification, excess Na^+ (140 – 193 mg L^{-1}) and suboptimal K^+ (54 – 81 mg L^{-1}) concentrations in treated PPW may induce salt stress in hydroponic lettuce and require targeted nutrient supplementation. However, the persistence of toxin-producing cyanobacteria such as *Limnothrix* highlights potential food safety risks.

Chapter 7: Conclusions and Recommendations

7.1 Summary

Section 1.4 outlined five specific research objectives aimed at assessing the feasibility of integrating poultry processing wastewater treatment with hydroponic crop production. Each objective has been addressed through pilot-scale experimentation, microbial analysis, nutrient analysis, lettuce yields and evaluation of foodborne pathogens. The food safety assessment in this dissertation specifically focused on pathogens commonly associated with PPW, such as *E. coli*, *Salmonella*, and *Campylobacter*, as immediate indicators of microbiological safety.

Objective 1: Evaluate the impact of microalgae on organics removal, nitrogen transformation, and fate of pathogens during long-term continuous treatment of PPW in pilot-scale bioreactors.

This study demonstrated a consistently high average sCOD removal (~88.0%) in the continuous bioreactor system treating poultry processing wastewater (both algal and bacterial treatment trains). Although sCOD removal was comparable, the algal bioreactors achieved slightly greater total nitrogen removal (20.5%) than the bacterial bioreactors (11.0%). Nitrification was limited in all bioreactors due to high organics content in PPW, short HRT, and DO constraints due to heterotrophic competition. However, hydroponic grow beds demonstrated robust nitrification (11.8 – 54.5 mg NO_3-N L^{-1}), supported by CO_2 supplementation and pH modulation. Throughout the treatment train, substantial reductions in microbial counts were observed at various stages, with no detectable *E. coli*, *Salmonella*, or *Campylobacter* in post-UV disinfection effluents. These findings confirm the feasibility of transforming PPW into a safe and nutrient-rich solution for hydroponic lettuce.

Objective 2: Determine the effects of treated PPW on lettuce yield and plant nutrient status and develop a nitrogen mass balance accounting for nitrogen inputs, losses, and plant uptake.

Lettuce cultivated solely on treated PPW showed a 58% average reduction in shoot growth, 5% average increase in root biomass and nutrient deficiencies (K, Mg, Ca, Cu) compared to mineral fertilizer controls. However, implementing pH control in the upstream bioreactors (H_2SO_4 dosing, pH=7.0) and downstream nutrient supplementation (N, P, K and micronutrients) improved physiological performance of lettuce irrigated with treated PPW to levels comparable to the hydroponic control. Nitrogen was not a limiting nutrient, as NUE ranged between 3.7% and 6.3%, with 65.4% to 83.0% of input nitrogen remaining in the effluents. This indicates the potential to support a larger hydroponic production as the nitrogen input (~213 g) could theoretically produce 30 – 38 kg of lettuce. Nevertheless, limitations in other nutrients (K, Mg, Ca, and Cu) highlight the need for targeted supplementation to prevent deficiencies and ensure optimal lettuce growth.

Objective 3: Assess system's response by quantifying the fate of *Salmonella* under high exogenous *Salmonella* influxes during simulated worst-case contamination scenarios and its implications for hydroponic lettuce.

The treatment system achieved 97.5 – 99.6% *Salmonella* removal in bioreactors with no detectable *Salmonella* in hydroponic lettuce when challenged exogenously with 3 $\log_{10}$ CFU mL^{-1} *Salmonella*. However, *Salmonella* removal efficiency in the bioreactor declined to 68.4% at 5 $\log_{10}$ CFU mL^{-1} dosage. Although enrichment methods suggested potential *Salmonella* persistence in post-UV treatment, there was no detection of *Salmonella* in hydroponic grow beds and lettuce. The system restored baseline pathogen levels once inoculation ceased, demonstrating the system's capability to manage worst-case *Salmonella* loads without advanced filtration technologies.

Objective 4: Investigate the impact of bioreactor operating conditions (illumination, CO_2 sparging, and pH control) on microbial community dynamics related to organics removal, nitrification, plant growth promotion, and food safety.

Bioreactor were dominated by Proteobacteria and Bacteroidetes, reflecting high heterotrophic activity and sCOD removal (>80%). *Desmodesmus* was the dominant green algae in illuminated bioreactors, while total nitrifier abundance remained low in all bioreactors (<0.03%) but increased in hydroponic beds (<4.31%) due to CO_2 sparging and favorable pH conditions. Acid-based pH control in bioreactors suppressed denitrification and favored DNRA pathways, resulting in nitrogen retention. Hydroponic beds enriched plant growth-promoting bacteria and siderophore activity (0.6 ng L^{-1} deferoxamine mesylate equivalents), but the co-occurrence of cyanobacteria and fungal taxa suggested risks of toxin accumulation and spoilage.

Objective 5: Implement a continuous multistage bioreactor configuration for upstream nitrification and examine the impact of hydraulic retention time on organics removal, nitrogen transformation, and microbial community dynamics under long-term operation.

The multistage bioreactor setup operating at 72-hr HRT sustained nitrification (~72% of total input nitrogen) and enhanced organics removal (80–96%) in all bioreactors. Algal bioreactors supported higher DO (~6.0 mg L^{-1}) and favorable redox conditions for early-stage nitrification. At 48-hr HRT, high loading rates suppressed nitrite oxidation, especially in bacterial bioreactors while enhancing nitrogen loss via denitrification. While sequential bioreactors improved nitrogen transformation, excess Na^+ and suboptimal K^+ levels in treated PPW may induce salt stress in lettuce and necessitate nutrient supplementation. The presence of *Limnothrix* and other potential toxin-producers highlight the importance of targeted management strategies to ensure food safety.

Overall, the multistage algal bioreactor configuration operated at a 48-hr HRT is recommended for optimal integration of poultry processing wastewater treatment with hydroponic crop production. Although the 72-hour HRT supported robust organic removal and complete nitrification, such extended retention times are impractical for real-world wastewater treatment applications. At the more feasible 48-hour HRT, the algal system outperformed the bacterial system in sustaining early-stage nitrification and superior nitrogen removal via denitrification, which are beneficial for hydroponic irrigation and for reducing effluent nitrogen loads, respectively. However, algal metabolites led to elevated residual soluble COD in treated effluent (~90 mg L^{-1}), which exceeds typical discharge limits (i.e., ≤10 mg L^{-1} $CBOD_5$ under Alabama NPDES standards) and would require further treatment prior to environmental discharge. Nonetheless, this trade-off may be manageable in closed-loop scenarios where discharge may not be immediate.

In contrast, the bacterial system may be preferable for operational simplicity and energy efficiency, as it delivered consistent organic removal and acceptable nitrification capacity. The bacterial system at 48-hour HRT achieved better sCOD removal (~54 mg L^{-1}) under the same conditions, offering a slight advantage for discharge compliance. However, it exhibited long-term nitrification failure due to declining nitrite oxidizer activity and nitrite accumulation, risking nitrite accumulation in hydroponic reservoirs which is undesirable due to pH incompatibility with lettuce.

7.2 Recommendations for future research

Although Poultryponics is promising for reusing PPW for sustainable food production, several limitations must be addressed to support its commercial implementation.

1. Impact of residual antimicrobials in PPW.

The impact of antimicrobial chemicals commonly used in poultry processing such as peracetic acid (PAA) and cetylpyridinium chloride (CPC) on microbial community dynamics, nitrogen cycling, and plant growth in long-term operation remains lacking.

2. Food safety, post-harvest quality and spoilage risk.

This dissertation only considered *E. coli*, *Salmonella* and *Campylobacter* as indicators of contamination of hydroponic lettuce. However, there is not enough information on cyanotoxin/mycotoxin production, spoilage risks, and post-harvest quality. Comprehensive assessment of these factors is essential to ensure safe and sustainable food production.

3. Life cycle assessment (LCA) and Techno-economic analysis (TEA).

In order transition to commercial scale, a full LCA and TEA are required. While this dissertation estimated nitrogen losses, it did not include direct measurement of gaseous emissions such as N_2 or N_2O, which are critical for assessing nitrogen fate and environmental impact. Future studies should aim to quantify the system's energy requirements, carbon and nitrogen footprints, capital and operational costs, and overall environmental impacts, enabling a robust comparison with conventional hydroponic systems and open-field agriculture.

4. Linking microbial community profiles to system functionality.

A critical challenge observed in this research was the mismatch between molecular characterizations (e.g., 16S/18S rDNA sequencing, qPCR, and functional prediction tools such as Tax4Fun2) and actual system performance. These discrepancies highlight the need to incorporate meta-transcriptomics to assess real-time microbial activity and metabolic functions in wastewater-based hydroponics.

5. Recommendation for industry and policy stakeholders

To advance the commercial viability of Poultryponics, integrated frameworks should be developed that link poultry processors, wastewater treatment facilities, and hydroponic growers under shared compliance and cost models. Such partnerships would facilitate coordinated management, ensure accountability, and streamline operational costs. Also, pilot-scale demonstration projects should be supported to validate economic feasibility, demonstrate scalability, and build market confidence. From a policy perspective, there is a need for harmonized national guidelines governing the reuse of treated wastewater in food crop irrigation, with specific provisions addressing the unique risks and opportunities associated with poultry effluents. Lastly, investment in demonstration plants and education campaigns can promote regulatory acceptance and bolster consumer trust, ensuring that innovations in circular water reuse are both safe and socially sustainable.

References

1. Food and Agricultural Organization of the United Nations (FAO). *How to Feed the World in 2050*.; 2009.
2. Alexandratos N, Bruinsma J. *World Agriculture towards 2030/2050: The 2012 Revision*.; 2012. www.fao.org/economic/esa
3. Mottet A, Tempio G. Global poultry production: current state and future outlook and challenges. *Worlds Poult Sci J*. 2017;73(2):245-256. doi:10.1017/S0043933917000071
4. Henchion M, Moloney AP, Hyland J, Zimmermann J, McCarthy S. Review: Trends for meat, milk and egg consumption for the next decades and the role played by livestock systems in the global production of proteins. *Animal*. 2021;15:100287. doi:10.1016/j.animal.2021.100287
5. Kiepper BH. *Characterization of Poultry Processing Operations, Wastewater Generation, and Wastewater Treatment Using Mail Survey and Nutrient Discharge Monitoring Methods*. 2003.
6. Bustillo-Lecompte CF, Mehrvar M. Slaughterhouse wastewater characteristics, treatment, and management in the meat processing industry: A review on trends and advances. *J Environ Manage*. 2015;161:287-302. doi:10.1016/j.jenvman.2015.07.008
7. Jayathilakan K, Sultana K, Radhakrishna K, Bawa AS. Utilization of byproducts and waste materials from meat, poultry and fish processing industries: a review. *J Food Sci Technol*. 2012;49(3):278-293. doi:10.1007/s13197-011-0290-7
8. Northcutt JK, Jones DR. A survey of water use and common industry practices in commercial broiler processing facilities. *Journal of Applied Poultry Research*. 2004;13(1):48-54. doi:10.1093/japr/13.1.48
9. National Agricultural Statistical Service (USDA). *Poultry - Production and Value*.; 2024.
10. Chen J, Pavlostathis SG. Peracetic acid fate and decomposition in poultry processing wastewater streams. *Bioresour Technol Rep*. 2019;7. doi:10.1016/j.biteb.2019.100285
11. Pilgrim's. Removing Nitrogen from Wastewater. 2023. Accessed June 1, 2025. https://sustainability.pilgrims.com/our-stories/environment/removing-nitrogen-from-wastewater/
12. Marel. Sustainable water use in the poultry industry, Marel Poultry, Qatar. 2019. https://www.agilitypr.news/Sustainable-water-use-in-the-poultry-ind-4562
13. Mittal GS. Characterization of the effluent wastewater from abattoirs for land application. *Food Reviews International*. 2004;20(3):229-256. doi:10.1081/FRI-200029422
14. Barros LSS, Amaral LA, Lorenzon CS, Junior JL, Neto JGM. Potential microbiological contamination of effluents in poultry and swine abattoirs. *Epidemiol Infect*. 2007;135(3):505-518. doi:10.1017/S0950268806006972
15. Wu X, Nawaz S, Li Y, Zhang H. Environmental health hazards of untreated livestock wastewater: potential risks and future perspectives. *Environmental Science and Pollution Research*. 2024;31(17):24745-24767. doi:10.1007/s11356-024-32853-6
16. Marshall KE, Hexemer A, Seelman SL, et al. Lessons Learned from a Decade of Investigations of Shiga Toxin-Producing Escherichia coli Outbreaks Linked to Leafy Greens, United States and Canada. *Emerg Infect Dis*. 2020;26(10):2319-2328. doi:10.3201/eid2610.191418
17. Carstens CK, Salazar JK, Darkoh C. Multistate Outbreaks of Foodborne Illness in the United States Associated With Fresh Produce From 2010 to 2017. *Front Microbiol*. 2019;10. doi:10.3389/fmicb.2019.02667

18. Centers for Disease Control and Prevention. Salmonella. 2024. https://www.cdc.gov/salmonella/index.html
19. Food and Agricultural Organization of the United Nations (FAO). *The State of Food Insecurity in the World 2011: How Does International Price Volatility Affect Domestic Economies and Food Security?* Food and Agriculture Organization of the United Nations; 2011.
20. Szekely I, Jijakli MH. Bioponics as a Promising Approach to Sustainable Agriculture: A Review of the Main Methods for Producing Organic Nutrient Solution for Hydroponics. *Water (Switzerland)*. 2022;14(23). doi:10.3390/w14233975
21. Wongkiew S, Park MR, Chandran K, Khanal SK. Aquaponic Systems for Sustainable Resource Recovery: Linking Nitrogen Transformations to Microbial Communities. *Environ Sci Technol*. 2018;52(21):12728-12739. doi:10.1021/acs.est.8b04177
22. Samoraj M, Mironiuk M, Izydorczyk G, et al. The challenges and perspectives for anaerobic digestion of animal waste and fertilizer application of the digestate. *Chemosphere*. 2022;295:133799. doi:10.1016/j.chemosphere.2022.133799
23. Zhang X, Davidson EA, Mauzerall DL, Searchinger TD, Dumas P, Shen Y. Managing nitrogen for sustainable development. *Nature*. 2015;528(7580):51-59. doi:10.1038/nature15743
24. Gartmann F, Hügly J, Krähenbühl N, et al. Bioponics—An Organic Closed-Loop Soilless Cultivation System: Yields and Characteristics Compared to Hydroponics and Soil Cultivation. *Agronomy*. 2023;13(6). doi:10.3390/agronomy13061436
25. Wongkiew S, Polprasert C, Koottatep T, Limpiyakorn T, Surendra KC, Khanal SK. Chicken manure-based bioponics: Effects of acetic acid supplementation on nitrogen and phosphorus recoveries and microbial communities. *Waste Management*. 2022;137:264-274. doi:10.1016/j.wasman.2021.11.023
26. Aksorn S, Kanokkantapong V, Polprasert C, Noophan P (Lek), Khanal SK, Wongkiew S. Effects of Cu and Zn contamination on chicken manure-based bioponics: Nitrogen recovery, bioaccumulation, microbial community, and health risk assessment. *J Environ Manage*. 2022;311. doi:10.1016/j.jenvman.2022.114837
27. Libutti A, Gatta G, Gagliardi A, et al. Agro-industrial wastewater reuse for irrigation of a vegetable crop succession under Mediterranean conditions. *Agric Water Manag*. 2018;196:1-14. doi:10.1016/j.agwat.2017.10.015
28. Elmeddahi Y, Mahmoudi H, Issaadi A, Goosen MFA. Analysis of treated wastewater and feasibility for reuse in irrigation: a case study from Chlef, Algeria. *Desalination Water Treat*. 2016;57(12):5222-5231. doi:10.1080/19443994.2015.1021999
29. Gatta G, Libutti A, Beneduce L, et al. Reuse of treated municipal wastewater for globe artichoke irrigation: Assessment of effects on morpho-quantitative parameters and microbial safety of yield. *Sci Hortic*. 2016;213:55-65. doi:10.1016/j.scienta.2016.10.011
30. Wdowikowska A, Reda M, Kabała K, et al. Water and Nutrient Recovery for Cucumber Hydroponic Cultivation in Simultaneous Biological Treatment of Urine and Grey Water. *Plants*. 2023;12(6). doi:10.3390/plants12061286
31. Goddek S, Delaide B, Mankasingh U, Ragnarsdottir KV, Jijakli H, Thorarinsdottir R. Challenges of sustainable and commercial aquaponics. *Sustainability (Switzerland)*. 2015;7(4):4199-4224. doi:10.3390/su7044199
32. Wongkiew S, Hu Z, Chandran K, Lee JW, Khanal SK. Nitrogen transformations in aquaponic systems: A review. *Aquac Eng*. 2017;76:9-19. doi:10.1016/j.aquaeng.2017.01.004

33. Love DC, Fry JP, Li X, et al. Commercial aquaponics production and profitability: Findings from an international survey. *Aquaculture*. 2015;435:67-74. doi:10.1016/j.aquaculture.2014.09.023
34. Gómez C, Currey CJ, Dickson RW, et al. Controlled Environment Food Production for Urban Agriculture. *HortScience*. 2019;54(9):1448-1458. doi:10.21273/HORTSCI14073-19
35. Mehra M, Saxena S, Sankaranarayanan S, Tom RJ, Veeramanikandan M. IoT based hydroponics system using Deep Neural Networks. *Comput Electron Agric*. 2018;155:473-486. doi:10.1016/j.compag.2018.10.015
36. Ramin Shamshiri R, Kalantari F, C. Ting K, et al. Advances in greenhouse automation and controlled environment agriculture: A transition to plant factories and urban agriculture. *International Journal of Agricultural and Biological Engineering*. 2018;11(1):1-22. doi:10.25165/j.ijabe.20181101.3210
37. Coskun T, Debik E, Kabuk HA, et al. Treatment of poultry slaughterhouse wastewater using a membrane process, water reuse, and economic analysis. *Desalination Water Treat*. 2016;57(11):4944-4951. doi:10.1080/19443994.2014.999715
38. Rosso D, Larson LE, Stenstrom MK. Aeration of large-scale municipal wastewater treatment plants: state of the art. *Water Science and Technology*. 2008;57(7):973-978. doi:10.2166/wst.2008.218
39. Metcalf & Eddy. *Wastewater Engineering: Treatment and Reuse*. Vol 4. 4th ed. McGraw-Hill Companies, Inc; 2003.
40. Fallahi A, Rezvani F, Asgharnejad H, Khorshidi E, Hajinajaf N, Higgins B. Interactions of microalgae-bacteria consortia for nutrient removal from wastewater: A review. *Chemosphere*. 2021;272. doi:10.1016/j.chemosphere.2021.129878
41. Bhatt A, Arora P, Prajapati SK. Chlorella pyrenoidosa-mediated removal of pathogenic bacteria from municipal wastewater – Multivariate process optimization and application in the real sewage. *J Environ Chem Eng*. 2023;11(2). doi:10.1016/j.jece.2023.109494
42. Ansa EDO, Awuah E, Andoh A, et al. A Review of the Mechanisms of Faecal Coliform Removal from Algal and Duckweed Waste Stabilization Pond Systems. *Am J Environ Sci*. 2015;11(1):28-34. doi:10.3844/AJESSP.2015.28.34
43. Wang Q, Higgins BT. Long Term Outdoor Algae Production on Undiluted Anaerobic Digestate in the Southeastern US. *Journal of the ASABE*. 2024;67(1):181-192. doi:10.13031/ja.15727
44. Franke-Whittle IH, Walter A, Ebner C, Insam H. Investigation into the effect of high concentrations of volatile fatty acids in anaerobic digestion on methanogenic communities. *Waste Management*. 2014;34(11):2080-2089. doi:10.1016/j.wasman.2014.07.020
45. Hernandez JE, Edyvean RGJ. Inhibition of biogas production and biodegradability by substituted phenolic compounds in anaerobic sludge. *J Hazard Mater*. 2008;160(1):20-28. doi:10.1016/j.jhazmat.2008.02.075
46. Bankston E, Wang Q, Higgins BT. Algae support populations of heterotrophic, nitrifying, and phosphate-accumulating bacteria in the treatment of poultry litter anaerobic digestate. *Chemical Engineering Journal*. 2020;398. doi:10.1016/j.cej.2020.125550
47. Wang Q, Childree E, Box J, et al. Microalgae Can Promote Nitrification in Poultry-Processing Wastewater in the Presence and Absence of Antimicrobial Agents. *ACS ES and T Engineering*. 2023;3(4):568-579. doi:10.1021/acsestengg.2c00360
48. Ferreira A, Marques P, Ribeiro B, et al. Combining biotechnology with circular bioeconomy: From poultry, swine, cattle, brewery, dairy and urban wastewaters to biohydrogen. *Environ Res*. 2018;164:32-38. doi:10.1016/j.envres.2018.02.007

49. Oliveira AC, Barata A, Batista AP, Gouveia L. Scenedesmus obliquus in poultry wastewater bioremediation. *Environ Technol*. 2019;40(28):3735-3744. doi:10.1080/09593330.2018.1488003
50. Spolaore P, Joannis-Cassan C, Duran E, Isambert A. Commercial applications of microalgae. *J Biosci Bioeng*. 2006;101(2):87-96. doi:10.1263/jbb.101.87
51. Hyman M, Wang Q, Wilson AE, Adhikari S, Higgins BT. Production of Daphnia zooplankton on wastewater-grown algae for sustainable conversion of waste nutrients to fish feed. *J Clean Prod*. 2021;310:127501. doi:10.1016/j.jclepro.2021.127501
52. Mauerer M, Rocksch T, Dannehl D, et al. Replacing Mineral Fertilizer with Nitrified Human Urine in Hydroponic Lettuce (Lactuca sativa L.) Production. *Sustainability (Switzerland)*. 2023;15(13). doi:10.3390/su151310684
53. United States Department of Agriculture. Foreign Agricultural Service. *Livestock and Poultry: World Markets and Trade. Weaker China Demand Shifts Global Trade*.; 2024.
54. Carawan R, Taylor MC, Curtis PA, Keener K. Survey Shows That Poultry Processors Can Save Money by Conserving Water. *Pollution Prevention Pays in Food Processing*. Published online 2014:3-4.
55. Koenraadl PMFJ, Ayling R, Hazelegeri WC, Rombouts't And FM, Newell DG. The speciation and subtyping of campylobacter isolates from sewage plants and waste water from a connected poultry abattoir using molecular techniques. *Epidemniol Infect*. 1995;115:485.
56. McClure M, Whitney B, Gardenhire I, et al. An Outbreak Investigation of Salmonella Typhimurium Illnesses in the United States Linked to Packaged Leafy Greens Produced at a Controlled Environment Agriculture Indoor Hydroponic Operation – 2021. *J Food Prot*. 2023;86(5). doi:10.1016/j.jfp.2023.100079
57. Nirmalakhandan N, Selvaratnam T, Henkanatte-Gedera SM, et al. Algal wastewater treatment: Photoautotrophic vs. mixotrophic processes. *Algal Res*. 2019;41. doi:10.1016/j.algal.2019.101569
58. Saravanan A, Kumar PS, Varjani S, et al. A review on algal-bacterial symbiotic system for effective treatment of wastewater. *Chemosphere*. 2021;271. doi:10.1016/j.chemosphere.2021.129540
59. Kee J, Lila C, Yacob Abrehe Zereyesus. Global Fertilizer Market Challenged by Russia's Invasion of Ukraine. *Amber Waves: The Economics of Food, Farming, Natural Resources, and Rural America 2023*. Published online September 18, 2023. http://ageconsearch.umn.edu
60. Magwaza ST, Magwaza LS, Odindo AO, Mditshwa A. Hydroponic technology as decentralised system for domestic wastewater treatment and vegetable production in urban agriculture: A review. *Science of the Total Environment*. 2020;698. doi:10.1016/j.scitotenv.2019.134154
61. Wongkiew S, Hu Z, Lee JW, et al. Nitrogen Recovery via Aquaponics–Bioponics: Engineering Considerations and Perspectives. *ACS ES&T Engineering*. 2021;1(3):326-339. doi:10.1021/acsestengg.0c00196
62. Södergren J, Larsson CU, Wadsö L, et al. Food waste to new food: Risk assessment and microbial community analysis of anaerobic digestate as a nutrient source in hydroponic production of vegetables. *J Clean Prod*. 2022;333. doi:10.1016/j.jclepro.2021.130239
63. Guruchandran S, Muninathan C, Ganesan ND. Novel strategy for effective utilization of anaerobic digestate as a nutrient medium for crop production in a recirculating deep water culture hydroponics system. *Biomass Convers Biorefin*. Published online 2022. doi:10.1007/s13399-022-03109-5

64. Egbuikwem PN, Mierzwa JC, Saroj DP. Assessment of suspended growth biological process for treatment and reuse of mixed wastewater for irrigation of edible crops under hydroponic conditions. *Agric Water Manag*. 2020;231. doi:10.1016/j.agwat.2020.106034
65. Wongkiew S, Koottatep T, Polprasert C, Prombutara P, Jinsart W, Khanal SK. Bioponic system for nitrogen and phosphorus recovery from chicken manure: Evaluation of manure loading and microbial communities. *Waste Management*. 2021;125:67-76. doi:10.1016/j.wasman.2021.02.014
66. Tsukamoto H, Phan H V., Suenaga T, et al. Microaerophilic Activated Sludge System for Ammonia Retention toward Recovery from High-Strength Nitrogenous Wastewater: Performance and Microbial Communities. *Environ Sci Technol*. 2023;57(37):13874-13886. doi:10.1021/acs.est.3c03002
67. Xu S, Wu X, Lu H. Overlooked nitrogen-cycling microorganisms in biological wastewater treatment. *Front Environ Sci Eng*. 2021;15(6). doi:10.1007/s11783-021-1426-2
68. Ansa EDO, Lubberding HJ, Ampofo JA, Gijzen HJ. The role of algae in the removal of Escherichia coli in a tropical eutrophic lake. *Ecol Eng*. 2011;37(2):317-324. doi:10.1016/j.ecoleng.2010.11.023
69. Jurga A, Ratkiewicz K, Wdowikowska A, et al. Urine and grey water based liquid fertilizer – Production and the response of plants. *J Environ Manage*. 2023;331. doi:10.1016/j.jenvman.2023.117248
70. Janiak K, Jurga A, Wizimirska A, et al. Urine nitrification robustness for application in space: Effect of high salinity and the response to extreme free ammonia concentrations. *J Environ Manage*. 2021;279. doi:10.1016/j.jenvman.2020.111610
71. Soroosh H, Otterpohl R, Hanelt D. Influence of supplementary carbon on reducing the hydraulic retention time in microalgae-bacteria (MaB) treatment of municipal wastewater. *Journal of Water Process Engineering*. 2023;51. doi:10.1016/j.jwpe.2022.103447
72. Wallace-Springer N, Wells DE, Pickens JM, Ayipio E, Kemble J. Effects of Hydraulic Retention Time of Aquaculture Effluent on Nutrient Film Technique Lettuce Productivity. *Agronomy*. 2022;12(10). doi:10.3390/agronomy12102570
73. Sonneveld C, Straver N. *Nutrient Solutions for Vegetables and Flowers Grown in Water or Substrates*. Vol 8. 10th ed.; 1994.
74. Wang Q, Cherones J, Higgins B. Acclimation of an algal consortium to sequester nutrients from anaerobic digestate. *Bioresour Technol*. 2021;342. doi:10.1016/j.biortech.2021.125921
75. Kim J, Oh E, Banting GS, et al. An improved culture method for selective isolation of Campylobacter jejuni from wastewater. *Front Microbiol*. 2016;7(AUG). doi:10.3389/fmicb.2016.01345
76. Tamir G, Shenker M, Heller H, Bloom PR, Fine P, Bar-Tal A. Organic N mineralization and transformations in soils treated with animal waste in relation to carbonate dissolution and precipitation. *Geoderma*. 2013;209-210:50-56. doi:10.1016/j.geoderma.2013.05.028
77. Tang G, Li B, Zhang B, et al. Dynamics of dissolved organic matter and dissolved organic nitrogen during anaerobic/anoxic/oxic treatment processes. *Bioresour Technol*. 2021;331. doi:10.1016/j.biortech.2021.125026
78. Xu G, Zhang Z, Gao F. Effect of COD/N ratios and DO concentrations on the NOB suppression in a multi-cycle SBR. *J Environ Chem Eng*. 2021;9(4). doi:10.1016/j.jece.2021.105735
79. Choi D, Jung J. Nitrogen removal enhancement through competitive inhibition of nitrite oxidizing bacteria in mainstream partial nitritation/anammox: Anammox seeding and influent C/N ratios. *Biochem Eng J*. 2023;195. doi:10.1016/j.bej.2023.108910

80. Regmi P, Miller MW, Holgate B, et al. Control of aeration, aerobic SRT and COD input for mainstream nitritation/denitritation. *Water Res*. 2014;57:162-171. doi:10.1016/j.watres.2014.03.035
81. Higgins BT, Gennity I, Fitzgerald PS, Ceballos SJ, Fiehn O, VanderGheynst JS. Algal–bacterial synergy in treatment of winery wastewater. *NPJ Clean Water*. 2018;1(1). doi:10.1038/s41545-018-0005-y
82. Flores-Salgado G, Thalasso F, Buitrón G, Vital-Jácome M, Quijano G. Kinetic characterization of microalgal-bacterial systems: Contributions of microalgae and heterotrophic bacteria to the oxygen balance in wastewater treatment. *Biochem Eng J*. 2021;165. doi:10.1016/j.bej.2020.107819
83. Osem Y, Chen Y, Levinson D, Hadar Y. The effects of plant roots on microbial community structure in aerated wastewater-treatment reactors. *Ecol Eng*. 2007;29(2):133-142. doi:10.1016/j.ecoleng.2006.06.003
84. Anthonisen AC, Loehr RC, Prakasam TB, Srinath EG. Inhibition of nitrification by ammonia and nitrous acid. *J Water Pollut Control Fed*. 1976;48(5):835-852.
85. Biesterfeld S, Farmer G, Russell P, Figueroa L. Effect of Alkalinity Type and Concentration on Nitrifying Biofilm Activity. *Source: Water Environment Research*. 2003;75(3):196-204. Accessed November 14, 2023. https://www.jstor.org/stable/25045684
86. Wett B, Rauch W. The role of inorganic carbon limitation in biological nitrogen removal of extremely ammonia concentrated wastewater. *Water Res*. 2003;37:1100-1110.
87. Denecke M, Liebig T. Effect of carbon dioxide on nitrification rates. *Bioprocess Biosyst Eng*. 2003;25(4):249-253. doi:10.1007/s00449-002-0303-z
88. Savvas D, Passam HC, Olympios C, et al. Effects of Ammonium Nitrogen on Lettuce Grown on Pumice in a Closed Hydroponic System. *HORTSCIENCE*. 2006;41(7):1667-1673.
89. Tzortzakis NG. Influence of NaCl and calcium nitrate on lettuce and endive growth using nutrient film technique. *International Journal of Vegetable Science*. 2009;15(1):44-56. doi:10.1080/19315260802446419
90. Adhikari B, Olorunwa OJ, Brazel S, Barickman TC, Bheemanahalli R. Impact of salt stress on physiology, leaf mass, and nutrient accumulation in romaine lettuce. *Photosynthetica*. 2023;61(3):342-353. doi:10.32615/ps.2023.027
91. Parida A, Das AB, Das P. NaCI Stress Causes Changes in Photosynthetic Pigments, Proteins, and Other Metabolic Components in the Leaves of a True Mangrove, Bruguiera parviflora, in Hydroponic Cultures. *Journal of Plant Biology*. 2002;45(1):28-36.
92. Gao H, Gong L, Ni J, Li Q. Metabolomics Analysis of Lettuce (Lactuca sativa L.) Affected by Low Potassium Supply. *Agriculture (Switzerland)*. 2022;12(8). doi:10.3390/agriculture12081153
93. Gao H, Mao H, Ullah I. Analysis of metabolomic changes in lettuce leaves under low nitrogen and phosphorus deficiencies stresses. *Agriculture (Switzerland)*. 2020;10(9):1-14. doi:10.3390/agriculture10090406
94. Ilic S, Moodispaw MR, Madden L V., Lewis Ivey ML. Lettuce Contamination and Survival of Salmonella Typhimurium and Listeria monocytogenes in Hydroponic Nutrient Film Technique Systems. *Foods*. 2022;11(21). doi:10.3390/foods11213508
95. Ndulini SF, Sithole GM, Mthembu MS. Investigation of nutrients and faecal coliforms removal in wastewater using a hydroponic system. *Physics and Chemistry of the Earth*. 2018;106:68-72. doi:10.1016/j.pce.2018.05.004

96. Nasser AM, Paulman H, Sela O, et al. UV disinfection of wastewater effluents for unrestricted irrigation. *Water Science and Technology*. 2006;54(3):83-88. doi:10.2166/wst.2006.452
97. Kalisvaart BF. Re-use of wastewater: preventing the recovery of pathogens by using medium-pressure UV lamp technology. *Water Science and Technology*. 2004;50(6):337-344. http://iwaponline.com/wst/article-pdf/50/6/337/419990/337.pdf
98. Parlasca MC, Qaim M. Meat Consumption and Sustainability. *Annu Rev Resour Economics*. 2022;14(1):17-41. doi:10.1146/annurev-resource-111820-032340
99. Gerbens-Leenes PW, Mekonnen MM, Hoekstra AY. The water footprint of poultry, pork and beef: A comparative study in different countries and production systems. *Water Resour Ind*. 2013;1-2:25-36. doi:10.1016/j.wri.2013.03.001
100. Arthur W, Morgan Z, Reina Antillon M, et al. Pilot-Scale Evaluation of Poultryponics: Insights into Nitrogen Utilization and Food Pathogen Dynamics. *ACS ES&T Water*. 2024;4(9):3964-3975. doi:10.1021/acsestwater.4c00262
101. Leip A, Leach A, Musinguzi P, et al. Nitrogen-neutrality: a step towards sustainability. *Environmental Research Letters*. 2014;9(11):115001. doi:10.1088/1748-9326/9/11/115001
102. Adrover M, Moyà G, Vadell J. Use of hydroponics culture to assess nutrient supply by treated wastewater. *J Environ Manage*. 2013;127:162-165. doi:10.1016/j.jenvman.2013.04.044
103. Ikeda H, Tan X. Urea as an organic nitrogen source for hydroponically grown tomatoes in comparison with inorganic nitrogen sources. *Soil Sci Plant Nutr*. 1998;44(4):609-615. doi:10.1080/00380768.1998.10414484
104. Zhang H, Yuan X, Xiong T, Wang H, Jiang L. Bioremediation of co-contaminated soil with heavy metals and pesticides: Influence factors, mechanisms and evaluation methods. *Chemical Engineering Journal*. 2020;398:125657. doi:10.1016/j.cej.2020.125657
105. Bergstrand KJ, Asp H, Hultberg M. Utilizing anaerobic digestates as nutrient solutions in hydroponic production systems. *Sustainability (Switzerland)*. 2020;12(23):1-12. doi:10.3390/su122310076
106. Holmes SC, Wells DE, Pickens JM, Kemble JM. Selection of heat tolerant lettuce (Lactuca sativa L.) cultivars grown in deep water culture and their marketability. *Horticulturae*. 2019;5(3). doi:10.3390/horticulturae5030050
107. Wallace-Springer N. *Effects of Hydraulic Retention Time on Nutrient Film Technique Lettuce Production in a Decoupled Aquaponics System*. Auburn University; 2020.
108. Erice G, Louahlia S, Irigoyen JJ, Sanchez-Diaz M, Avice JC. Biomass partitioning, morphology and water status of four alfalfa genotypes submitted to progressive drought and subsequent recovery. *J Plant Physiol*. 2010;167(2):114-120. doi:10.1016/j.jplph.2009.07.016
109. Elliott GC, Läuchli A. Phosphorus Efficiency and Phosphate–Iron Interaction in Maize. *Agron J*. 1985;77(3):399-403. doi:10.2134/agronj1985.00021962007700030011x
110. Siddiqi MY, Glass ADM. Utilization index: A modified approach to the estimation and comparison of nutrient utilization efficiency in plants. *J Plant Nutr*. 1981;4(3):289-302. doi:10.1080/01904168109362919
111. Ríos JJ, Blasco B, Cervilla LM, et al. Nitrogen-use efficiency in relation to different forms and application rates of Se in lettuce plants. *J Plant Growth Regul*. 2010;29(2):164-170. doi:10.1007/s00344-009-9130-7
112. Leone C, Xu X, Mishra A, Thippareddi H, Singh M. Interventions to reduce Salmonella and Campylobacter during chilling and post-chilling stages of poultry processing: a systematic review and meta-analysis. *Poult Sci*. 2024;103(4). doi:10.1016/j.psj.2024.103492

113. Li Y, Zhou C, Wang S, et al. Phytotoxicity and oxidative effects of typical quaternary ammonium compounds on wheat (Triticum aestivum L.) seedlings. *Environmental Science and Pollution Research*. 2019;26(25):25985-25999. doi:10.1007/s11356-019-05822-7
114. Marth PC, Preston , William H., Mitchell JW. Growth-Controlling Effects of some Quaternary Ammonium Compounds on Various Species of Plants. *Botanical Gazette*. 1953;115(2):200-204. doi:10.1086/335816
115. Wang L, Guo S, Wang Y, Yi D, Wang J. Poultry biogas slurry can partially substitute for mineral fertilizers in hydroponic lettuce production. *Environmental Science and Pollution Research*. 2019;26(1):659-671. doi:10.1007/s11356-018-3538-1
116. Vaillant N, Monnet F, Sallanon H, Coudret A, Hitmi A. Use of Commercial Plant Species in a Hydroponic System to Treat Domestic Wastewaters. *J Environ Qual*. 2004;33(2):695-702. doi:10.2134/jeq2004.6950
117. Jin E, Cao L, Xiang S, Zhou W, Ruan R, Liu Y. Feasibility of using pretreated swine wastewater for production of water spinach (Ipomoea aquatic Forsk.) in a hydroponic system. *Agric Water Manag*. 2020;228. doi:10.1016/j.agwat.2019.105856
118. Lawlor DW. Mengel, K. and Kirkby, E. A. Principles of plant nutrition. *Ann Bot*. 2004;93(4):479-480. doi:10.1093/aob/mch063
119. Yadav RK, Sahoo S, Yadav AK, Patil SA. Epipremnum aureum is a promising plant candidate for developing nature-based technologies for nutrients removal from wastewaters. *J Environ Chem Eng*. 2021;9(5). doi:10.1016/j.jece.2021.106134
120. Woods J, Williams A, Hughes JK, Black M, Murphy R. Energy and the food system. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 2010;365(1554):2991-3006. doi:10.1098/rstb.2010.0172
121. Alejandro S, Höller S, Meier B, Peiter E. Manganese in Plants: From Acquisition to Subcellular Allocation. *Front Plant Sci*. 2020;11. doi:10.3389/fpls.2020.00300
122. Singh H, Bruce D. *Electrical Conductivity and pH Guide for Hydroponics*.; 2016. http://osufacts.okstate.edu
123. Maggio A, Raimondi G, Martino A, De Pascale S. Salt stress response in tomato beyond the salinity tolerance threshold. *Environ Exp Bot*. 2007;59(3):276-282. doi:10.1016/j.envexpbot.2006.02.002
124. Savvas D, Passam HC, Olympios C, et al. Effects of Ammonium Nitrogen on Lettuce Grown on Pumice in a Closed Hydroponic System. *HORTSCIENCE*. 2006;41(7):1667-1673.
125. Wang M, Dong C, Gao W. Evaluation of the growth, photosynthetic characteristics, antioxidant capacity, biomass yield and quality of tomato using aeroponics, hydroponics and porous tube-vermiculite systems in bio-regenerative life support systems. *Life Sci Space Res (Amst)*. 2019;22:68-75. doi:10.1016/j.lssr.2019.07.008
126. Nancharaiah YV, Kiran Kumar Reddy G. Aerobic granular sludge technology: Mechanisms of granulation and biotechnological applications. *Bioresour Technol*. 2018;247:1128-1143. doi:10.1016/j.biortech.2017.09.131
127. Wongkiew S, Polprasert C, Noophan P (Lek), et al. Effects of vermicompost leachate on nitrogen, phosphorus, and microbiome in a food waste bioponic system. *J Environ Manage*. 2023;339. doi:10.1016/j.jenvman.2023.117860
128. Scheierling SM, Bartone C, Mara DD, Drechsel P. *Improving Wastewater Use in Agriculture: An Emerging Priority*.; 2010.
129. Centers for Disease Control and Prevention. Lettuce, other Leafy Greens, and Food Safety. 2024. https://www.cdc.gov/foodsafety/communication/leafy-greens.html#

130. Weller DL, Saylor L, Turkon P. Total coliform and generic E. Coli levels, and salmonella presence in eight experimental aquaponics and hydroponics systems: A brief report highlighting exploratory data. *Horticulturae*. 2020;6(3):1-9. doi:10.3390/horticulturae6030042
131. Wang YJ, Deering AJ, Kim HJ. The occurrence of Shiga toxin-producing E. Coli in aquaponic and hydroponic systems. *Horticulturae*. 2020;6(1). doi:10.3390/horticulturae6010001
132. Fatima F, Fatima S, Du H, Kommalapati RR. An Evaluation of Microfiltration and Ultrafiltration Pretreatment on the Performance of Reverse Osmosis for Recycling Poultry Slaughterhouse Wastewater. *Separations*. 2024;11(4):115. doi:10.3390/separations11040115
133. Folorunso EA, Schmautz Z, Gebauer R, Mraz J. The economic viability of commercial-scale hydroponics: Nigeria as a case study. *Heliyon*. 2023;9(8):e18979. doi:10.1016/j.heliyon.2023.e18979
134. Ravva S V., Sarreal CZ. Survival of salmonella enterica in aerated and nonaerated wastewaters from dairy lagoons. *Int J Environ Res Public Health*. 2014;11(11):11249-11260. doi:10.3390/ijerph111111249
135. Sánchez-Monedero MA, Aguilar MI, Fenoll R, Roig A. Effect of the aeration system on the levels of airborne microorganisms generated at wastewater treatment plants. *Water Res*. 2008;42(14):3739-3744. doi:10.1016/j.watres.2008.06.028
136. Szekely I, Zeaiter Z, Jijakli MH. Development of a Simple Bioponic Method Using Manure and Offering Comparable Lettuce Yield than Hydroponics. *Water (Switzerland)*. 2023;15(13). doi:10.3390/w15132335
137. Jurga A, Janiak K, Wizimirska A, et al. Resource recovery from synthetic nitrified urine in the hydroponic cultivation of lettuce (Lactuca sativa var. capitata l.). *Agronomy*. 2021;11(11). doi:10.3390/agronomy11112242
138. Topalcengiz Z, Mcegan R, Danyluk MD. Fate of salmonella in central Florida surface waters and evaluation of EPA worst case water as a standard medium. *J Food Prot*. 2019;82(6):916-925. doi:10.4315/0362-028X.JFP-18-331
139. Sela Saldinger S, Rodov V, Kenigsbuch D, Bar-Tal A. Hydroponic Agriculture and Microbial Safety of Vegetables: Promises, Challenges, and Solutions. *Horticulturae*. 2023;9(1). doi:10.3390/horticulturae9010051
140. Riggio GM, Jones SL, Gibson KE. Risk of human pathogen internalization in leafy vegetables during lab-scale hydroponic cultivation. *Horticulturae*. 2019;5(1). doi:10.3390/horticulturae5010025
141. Northcutt JK, Smith D, Huezo RI, Ingram KD. Microbiology of broiler carcasses and chemistry of chiller water as affected by water reuse. *Poult Sci*. 2008;87(7):1458-1463. doi:10.3382/ps.2007-00480
142. Berrang ME, Bailey JS. On-line brush and spray washers to lower numbers of Campylobacter and Escherichia coli and presence of Salmonella on broiler carcasses during processing. *Journal of Applied Poultry Research*. 2009;18(1):74-78. doi:10.3382/japr.2008-00067
143. *An Atlas of Salmonella in the United States, 1968 - 2011: Laboratory-Based Enteric Disease Surveillance*.; 2013.
144. Urrutia A, Orellana L, Sierra KS, et al. In vitro Effect of Photoactive Compounds Curcumin and Chlorophyllin Against Single Strains of Salmonella and Campylobacter. *J Food Prot*. 2023;86(11). doi:10.1016/j.jfp.2023.100157
145. Reina M, Urrutia A, Figueroa JC, et al. Application of pressurized steam and forced hot air for cleaning broiler transport container flooring. *Poult Sci*. 2024;103(2). doi:10.1016/j.psj.2023.103276

146. Mohammad ZH, Prado I do, Sirsat SA. Comparative microbial analyses of hydroponic versus in-soil grown Romaine lettuce obtained at retail. *Heliyon*. 2022;8(10):e11050. doi:10.1016/j.heliyon.2022.e11050
147. Andrews WH, Wang H, Jacobson A, Ge B, Zhang G, Hammack T. *Bacteriological Analytical Manual (BAM)- Chapter 5: Salmonella (April 2023 Edition)*.; 2023. https://www.fda.gov/food/laboratory-methods-food/bacteriological-analytical-manual-bam
148. Chen X, Bauermeister LJ, Hill GN, Singh M, Bilgili SF, McKee SR. Efficacy of various antimicrobials on reduction of Salmonella and Campylobacter and quality attributes of ground chicken obtained from poultry parts treated in a postchill decontamination tank. *J Food Prot*. 2014;77(11):1882-1888. doi:10.4315/0362-028X.JFP-14-114
149. Topalcengiz Z, Mcegan R, Danyluk MD. Fate of salmonella in central Florida surface waters and evaluation of EPA worst case water as a standard medium. *J Food Prot*. 2019;82(6):916-925. doi:10.4315/0362-028X.JFP-18-331
150. Chandran A, Hatha AAM. Relative survival of Escherichia coli and Salmonella typhimurium in a tropical estuary. *Water Res*. 2005;39(7):1397-1403. doi:10.1016/j.watres.2005.01.010
151. Munasinghe-Arachchige SP, Delanka-Pedige HMK, Henkanatte-Gedera SM, Tchinda D, Zhang Y, Nirmalakhandan N. Factors contributing to bacteria inactivation in the Galdieria sulphuraria-based wastewater treatment system. *Algal Res*. 2019;38. doi:10.1016/j.algal.2018.101392
152. Ansa EDO, Lubberding HJ, Gijzen HJ. The effect of algal biomass on the removal of faecal coliform from domestic wastewater. *Appl Water Sci*. 2012;2(2):87-94. doi:10.1007/s13201-011-0025-y
153. Shaima AF, Mohd Yasin NH, Ibrahim N, Takriff MS, Gunasekaran D, Ismaeel MYY. Unveiling antimicrobial activity of microalgae Chlorella sorokiniana (UKM2), Chlorella sp. (UKM8) and Scenedesmus sp. (UKM9). *Saudi J Biol Sci*. 2022;29(2):1043-1052. doi:10.1016/j.sjbs.2021.09.069
154. Moore-Neibel K, Gerber C, Patel J, Friedman M, Jaroni D, Ravishankar S. Antimicrobial activity of oregano oil against antibiotic-resistant Salmonella enterica on organic leafy greens at varying exposure times and storage temperatures. *Food Microbiol*. 2013;34(1):123-129. doi:10.1016/j.fm.2012.12.001
155. Dev Kumar G, Ravishankar S, Juneja VK. Interventions for Fresh Produce. In: *Microbial Control and Food Preservation*. Springer New York; 2017:199-223. doi:10.1007/978-1-4939-7556-3_10
156. Dev Kumar G, Ravishankar S. Ozonized water with plant antimicrobials: An effective method to inactivate Salmonella enterica on iceberg lettuce in the produce wash water. *Environ Res*. 2019;171:213-217. doi:10.1016/j.envres.2018.11.023
157. Barba FJ, Koubaa M, do Prado-Silva L, Orlien V, Sant'Ana A de S. Mild processing applied to the inactivation of the main foodborne bacterial pathogens: A review. *Trends Food Sci Technol*. 2017;66:20-35. doi:10.1016/j.tifs.2017.05.011
158. Mecha AC, Onyango MS, Ochieng A, Momba MNB. UV and solar photocatalytic disinfection of municipal wastewater: inactivation, reactivation and regrowth of bacterial pathogens. *International Journal of Environmental Science and Technology*. 2019;16(7):3687-3696. doi:10.1007/s13762-018-1950-1
159. Wang M, Ateia M, Awfa D, Yoshimura C. Regrowth of bacteria after light-based disinfection — What we know and where we go from here. *Chemosphere*. 2021;268. doi:10.1016/j.chemosphere.2020.128850

160. Shafaei S, Klamerth N, Zhang Y, McPhedran K, Bolton JR, Gamal El-Din M. Impact of environmental conditions on bacterial photoreactivation in wastewater effluents. *Environ Sci Process Impacts*. 2017;19(1):31-37. doi:10.1039/c6em00501b
161. Gayán E, Serrano MJ, Raso J, Álvarez I, Condón S. Inactivation of Salmonella enterica by UV-C light Alone and in combination with mild temperatures. *Appl Environ Microbiol*. 2012;78(23):8353-8361. doi:10.1128/AEM.02010-12
162. Leonardo Iniguez A, Dong Y, Carter HD, Ahmer BMM, Stone JM, Triplett EW. Regulation of Enteric Endophytic Bacterial Colonization by Plant Defenses. *Molecular Plant-Microbe Interactions*. 2005;18(2):169-178. doi:10.1094/MPMI
163. Schikora A, Carreri A, Charpentier E, Hirt H. The dark side of the salad: Salmonella typhimurium overcomes the innate immune response of arabidopsis thaliana and shows an endopathogenic lifestyle. *PLoS One*. 2008;3(5). doi:10.1371/journal.pone.0002279
164. Kroupitski Y, Golberg D, Belausov E, et al. Internalization of Salmonella enterica in leaves is induced by light and involves chemotaxis and penetration through open stomata. *Appl Environ Microbiol*. 2009;75(19):6076-6086. doi:10.1128/AEM.01084-09
165. Liu H, Whitehouse CA, Li B. Presence and Persistence of Salmonella in Water: The Impact on Microbial Quality of Water and Food Safety. *Front Public Health*. 2018;6. doi:10.3389/fpubh.2018.00159
166. Li D, Zeng S, Gu AZ, He M, Shi H. Inactivation, reactivation and regrowth of indigenous bacteria in reclaimed water after chlorine disinfection of a municipal wastewater treatment plant. *J Environ Sci (China)*. 2013;25(7):1319-1325. doi:10.1016/S1001-0742(12)60176-4
167. Daims H, Lebedeva E V., Pjevac P, et al. Complete nitrification by Nitrospira bacteria. *Nature*. 2015;528(7583):504-509. doi:10.1038/nature16461
168. Hachiya T, Watanabe CK, Fujimoto M, et al. Nitrate addition alleviates ammonium toxicity without lessening ammonium accumulation, organic acid depletion and inorganic cation depletion in arabidopsis thaliana shoots. *Plant Cell Physiol*. 2012;53(3):577-591. doi:10.1093/pcp/pcs012
169. Lorenzen J, Larsen LH, Kjær T, Revsbech NP. Biosensor Determination of the Microscale Distribution of Nitrate, Nitrate Assimilation, Nitrification, and Denitrification in a Diatom-Inhabited Freshwater Sediment. *Appl Environ Microbiol*. 1998;64(9):3264-3269. doi:10.1128/AEM.64.9.3264-3269.1998
170. An S, Joye SB. Enhancement of coupled nitrification-denitrification by benthic photosynthesis in shallow estuarine sediments. *Limnol Oceanogr*. 2001;46(1):62-74. doi:10.4319/lo.2001.46.1.0062
171. Gomez J, Mendez R, Lema JM. Kinetic Study of Addition of Volatile Organic Compounds to a Nitrifying Sludge. *Appl Biochem Biotechnol*. 2000;87(3):189-202. doi:10.1385/ABAB:87:3:189
172. van den Berg EM, Boleij M, Kuenen JG, Kleerebezem R, van Loosdrecht MCM. DNRA and Denitrification Coexist over a Broad Range of Acetate/N-NO3− Ratios, in a Chemostat Enrichment Culture. *Front Microbiol*. 2016;7. doi:10.3389/fmicb.2016.01842
173. Wang Z, Bu C, Dou J, et al. Enrichment of DNRA bacteria: Shift of microbial community and its combination with anammox to promote TN removal. *J Environ Chem Eng*. 2022;10(6):108867. doi:10.1016/j.jece.2022.108867
174. Arthur W, Morgan Z, Inskeep AE, et al. Assessing nitrogen recovery in poultryponics for hydroponic lettuce production using treated poultry processing wastewater for increased nitrogen neutrality. *Bioresour Technol*. 2025;422:132227. doi:10.1016/j.biortech.2025.132227

175. Mei C, Zhou D, Chretien RL, et al. A Potential Application of Pseudomonas psychrotolerans IALR632 for Lettuce Growth Promotion in Hydroponics. *Microorganisms*. 2023;11(2):376. doi:10.3390/microorganisms11020376
176. Buturi CV, Sabatino L, Mauro RP, et al. Iron Biofortification of Greenhouse Soilless Lettuce: An Effective Agronomic Tool to Improve the Dietary Mineral Intake. *Agronomy*. 2022;12(8):1793. doi:10.3390/agronomy12081793
177. Spaepen S, Das F, Luyten E, Michiels J, Vanderleyden J. Indole-3-acetic acid-regulated genes in Rhizobium etli CNPAF512. *FEMS Microbiol Lett*. 2009;291(2):195-200. doi:10.1111/j.1574-6968.2008.01453.x
178. Richardson AE, Barea JM, McNeill AM, Prigent-Combaret C. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant Soil*. 2009;321(1-2):305-339. doi:10.1007/s11104-009-9895-2
179. Stegelmeier AA, Rose DM, Joris BR, Glick BR. The Use of PGPB to Promote Plant Hydroponic Growth. *Plants*. 2022;11(20):2783. doi:10.3390/plants11202783
180. Wongkiew S, Amnuaychaichana S, Srithadindang K, et al. Enhancing Digestate-Based Bioponics through Ceramsite Addition with and without Biochar: Effects on Water Quality, Nutrient Recovery, Heavy Metal Removal, and Microbial Community Composition. *ACS ES&T Engineering*. Published online June 13, 2025. doi:10.1021/acsestengg.5c00263
181. Wang Q, Peng H, Higgins BT. Cultivation of Green Microalgae in Bubble Column Photobioreactors and an Assay for Neutral Lipids. *Journal of Visualized Experiments*. 2019;(143). doi:10.3791/59106
182. Schwyn B, Neilands JB. Universal chemical assay for the detection and determination of siderophores. *Anal Biochem*. 1987;160(1):47-56. doi:10.1016/0003-2697(87)90612-9
183. Zürcher E, Tavor-Deslex D, Lituiev D, Enkerli K, Tarr PT, Müller B. A Robust and Sensitive Synthetic Sensor to Monitor the Transcriptional Output of the Cytokinin Signaling Network in Planta . *Plant Physiol*. 2013;161(3):1066-1075. doi:10.1104/pp.112.211763
184. Khanna RR, Hasannin O, Thennakoon M, et al. trans-Zeatin N-glucosides can delay salt accelerated leaf senescence in Arabidopsis thaliana. *Plant Physiology and Biochemistry*. 2025;227:110101. doi:10.1016/j.plaphy.2025.110101
185. Oksanen J, Gavin L. Simpson, F. Guillaume Blanchet, et al. *Ordination Methods, Diversity Analysis and Other for Community and Vegetation Ecologists. Vegan: Community Ecology Package*. 2.6-10. Comprehensive R Archive Network; 2025. https://github.com/vegandevs/vegan
186. Wang R, Zhang H, Sun L, Qi G, Chen S, Zhao X. Microbial community composition is related to soil biological and chemical properties and bacterial wilt outbreak. *Sci Rep*. 2017;7(1):343. doi:10.1038/s41598-017-00472-6
187. Han L, Zhang H, Long Z, et al. Exploring microbial community structure and biological function in manured soil during ten repeated treatments with chlortetracycline and ciprofloxacin. *Chemosphere*. 2019;228:469-477. doi:10.1016/j.chemosphere.2019.04.154
188. Liang JL, Liu J, Jia P, et al. Novel phosphate-solubilizing bacteria enhance soil phosphorus cycling following ecological restoration of land degraded by mining. *ISME Journal*. 2020;14(6):1600-1613. doi:10.1038/s41396-020-0632-4
189. Magne F, Gotteland M, Gauthier L, et al. The Firmicutes/Bacteroidetes Ratio: A Relevant Marker of Gut Dysbiosis in Obese Patients? *Nutrients*. 2020;12(5):1474. doi:10.3390/nu12051474

190. Leta S, Gumaelius L, Assefa F, Dalhammar G. Identification of Efficient Denitrifying Bacteria from Tannery Wastewaters in Ethiopia and a Study of the Effects of Chromium III and Sulphide on Their Denitrification Rate. *World J Microbiol Biotechnol*. 2004;20(4):405-411. doi:10.1023/B:WIBI.0000033069.24982.6e
191. Harms H, Schlosser D, Wick LY. Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. *Nat Rev Microbiol*. 2011;9(3):177-192. doi:10.1038/nrmicro2519
192. Olicón-Hernández DR, González-López J, Aranda E. Overview on the Biochemical Potential of Filamentous Fungi to Degrade Pharmaceutical Compounds. *Front Microbiol*. 2017;8. doi:10.3389/fmicb.2017.01792
193. Suurnäkki S, Gomez-Saez G V., Rantala-Ylinen A, Jokela J, Fewer DP, Sivonen K. Identification of geosmin and 2-methylisoborneol in cyanobacteria and molecular detection methods for the producers of these compounds. *Water Res*. 2015;68:56-66. doi:10.1016/j.watres.2014.09.037
194. Zamora-Barrios CA, Nandini S, Sarma SSS. Review of potentially harmful cyanobacteria. *Tecnología y ciencias del agua*. 2023;14(3):250-313. doi:10.24850/j-tyca-14-03-06
195. Ahmad T, Belwal T, Li L, et al. Utilization of wastewater from edible oil industry, turning waste into valuable products: A review. *Trends Food Sci Technol*. 2020;99:21-33. doi:10.1016/j.tifs.2020.02.017
196. Mar CC, Fan Y, Li FL, Hu GR. Bioremediation of wastewater from edible oil refinery factory using oleaginous microalga Desmodesmus sp. S1. *Int J Phytoremediation*. 2016;18(12):1195-1201. doi:10.1080/15226514.2016.1193466
197. Basitere M, Rinquest Z, Njoya M, Sheldon MS, Ntwampe SKO. Treatment of poultry slaughterhouse wastewater using a static granular bed reactor (SGBR) coupled with ultrafiltration (UF) membrane system. *Water Science and Technology*. 2017;76(1):106-114. doi:10.2166/wst.2017.179
198. Eze CN, Ogbonna IO, Aoyagi H, Ogbonna JC. Effects of organic carbon sources on growth and oil accumulation by Desmodesmus subspicatus LC172266 under mixotrophic condition. *Arch Microbiol*. 2022;204(9):553. doi:10.1007/s00203-022-03165-5
199. Lazaratou CV, Traka K, Kaltsonoudis C, Tekerlekopoulou AG, Pandis SN, Vayenas D V. Dynamics of cyanobacteria/ bacterial consortia for CO2 fixation and simultaneous wastewater treatment. *J Environ Chem Eng*. 2024;12(6). doi:10.1016/j.jece.2024.114731
200. Cañizares-Villanueva RO, Ramos A, Corona AI, et al. Phormidium treatment of anaerobically treated swine wastewater. *Water Res*. 1994;28(9):1891-1895.
201. Wood SA, Puddick J, Fleming R, Heussner AH. Detection of anatoxin-producing Phormidium in a New Zealand farm pond and an associated dog death. *N Z J Bot*. 2017;55(1):36-46. doi:10.1080/0028825X.2016.1231122
202. Meincke M, Krieg E, Bock E. Nitrosovibrio spp., the Dominant Ammonia-Oxidizing Bacteria in Building Sandstone. *Appl Environ Microbiol*. 1989;55(8):2108-2110. doi:10.1128/aem.55.8.2108-2110.1989
203. Balkwill DL, Fredrickson JK, Romine MF. Sphingomonas and Related Genera. In: *The Prokaryotes*. Springer New York; 2006:605-629. doi:10.1007/0-387-30747-8_23
204. Arthur W, Akplah CK, Drabold E, et al. Dosing Salmonella into poultryponics: Fate of Salmonella during treatment of poultry processing wastewater and irrigation of hydroponic lettuce. *J Environ Manage*. 2025;377:124559. doi:10.1016/j.jenvman.2025.124559

205. Corrêa ÉB, Bettiol W, Morandi MAB. Biological control of Pythium aphanidermatum root rot and growth promotion of hydroponic lettuce by Clonostachys rosea. *Trop Plant Pathol*. 2010;35(4):248-252. doi:10.1590/S1982-56762010000400008
206. Card S, Jaspers MV, Walter M, Stewart A. Evaluation of microorganisms for biocontrol of grey mould on lettuce. *New Zealand Plant Protection*. 2002;55:197-201. doi:10.30843/nzpp.2002.55.3994
207. Haghpanah M, Jelodar NB, Zarrini HN, Pakdin-Parizi A, Dehestani A. New insights into azelaic acid-induced resistance against Alternaria Solani in tomato plants. *BMC Plant Biol*. 2024;24(1):687. doi:10.1186/s12870-024-05397-7
208. Campos-Cuevas JC, Pelagio-Flores R, Raya-González J, Méndez-Bravo A, Ortiz-Castro R, López-Bucio J. Tissue culture of Arabidopsis thaliana explants reveals a stimulatory effect of alkamides on adventitious root formation and nitric oxide accumulation. *Plant Science*. 2008;174(2):165-173. doi:10.1016/j.plantsci.2007.11.003
209. Romanis CS, Pearson LA, Neilan BA. Cyanobacterial blooms in wastewater treatment facilities: Significance and emerging monitoring strategies. *J Microbiol Methods*. 2021;180:106123. doi:10.1016/j.mimet.2020.106123
210. Pandey AK, Samota MK, Kumar A, Silva AS, Dubey NK. Fungal mycotoxins in food commodities: present status and future concerns. *Front Sustain Food Syst*. 2023;7. doi:10.3389/fsufs.2023.1162595
211. Tournas VH. Spoilage of vegetable crops by bacteria and fungi and related health hazards. *Crit Rev Microbiol*. 2005;31(1):33-44. doi:10.1080/10408410590886024
212. Solorzano-Morales A, Dolz G. Molecular characterization of Chlamydia species in commercial and backyard poultry farms in Costa Rica. *Epidemiol Infect*. 2022;150. doi:10.1017/S0950268821002715
213. Shanmugam SR, Schorer R, Arthur W, Drabold E, Rudar M, Higgins B. Upcycling nutrients from poultry slaughterhouse wastewater through cultivation of the nutritional yeast, Yarrowia lipolytica. *J Environ Chem Eng*. 2025;13(1):115245. doi:10.1016/j.jece.2024.115245
214. Aziz A, Basheer F, Sengar A, Irfanullah, Khan SU, Farooqi IH. Biological wastewater treatment (anaerobic-aerobic) technologies for safe discharge of treated slaughterhouse and meat processing wastewater. *Science of The Total Environment*. 2019;686:681-708. doi:10.1016/j.scitotenv.2019.05.295
215. Kassab G, Halalsheh M, Klapwijk A, Fayyad M, van Lier JB. Sequential anaerobic–aerobic treatment for domestic wastewater – A review. *Bioresour Technol*. 2010;101(10):3299-3310. doi:10.1016/j.biortech.2009.12.039
216. Soroosh H, Otterpohl R, Hanelt D. Influence of supplementary carbon on reducing the hydraulic retention time in microalgae-bacteria (MaB) treatment of municipal wastewater. *Journal of Water Process Engineering*. 2023;51:103447. doi:10.1016/j.jwpe.2022.103447
217. Yan X, Peng Y, Zhao Q, Zhang L, Liu J. Robust nitrogen removal via nitrification-partial denitrification / anammox to co-treat acrylic fiber wastewater and sewage without external carbon. *Chemical Engineering Journal*. 2024;498:155859. doi:10.1016/j.cej.2024.155859
218. Wang Z, Liu Z, Wang J, et al. Characterizing algal-bacterial symbiotic biofilms: Insights into coexistence of algae and anaerobic microorganisms. *Bioresour Technol*. 2024;406:130966. doi:10.1016/j.biortech.2024.130966
219. Fu W, Song G, Wang Y, et al. Advances in Research Into and Applications of Heterotrophic Nitrifying and Aerobic Denitrifying Microorganisms. *Front Environ Sci*. 2022;10. doi:10.3389/fenvs.2022.887093

220. Wang F, Yang K, Jiang W, et al. Factors affecting the enrichment of heterotrophic nitrifying aerobic denitrifying bacteria in municipal wastewater treatment systems and analysis of differences in nitrogen metabolism. *Journal of Water Process Engineering*. 2024;58. doi:10.1016/j.jwpe.2024.104807
221. Elefsiniotis P, Wareham DG, Smith MO. Use of volatile fatty acids from an acid-phase digester for denitrification. *J Biotechnol*. 2004;114(3):289-297. doi:10.1016/j.jbiotec.2004.02.016
222. Wongkiew S, Aksorn S, Amnuaychaichana S, et al. Bioponic systems with biochar: Insights into nutrient recovery, heavy metal reduction, and microbial interactions in digestate-based bioponics. *Waste Management*. 2024;178:267-279. doi:10.1016/j.wasman.2024.02.027
223. Janiak K, Jurga A, Wizimirska A, et al. Urine nitrification robustness for application in space: Effect of high salinity and the response to extreme free ammonia concentrations. *J Environ Manage*. 2021;279. doi:10.1016/j.jenvman.2020.111610
224. Sprague D. *Solids Retention Governs Nitrification of Poultry Processing Wastewater Using an Algal-Bacterial Consortium*. Auburn University; 2021.
225. Chaump K, Preisser M, Shanmugam SR, Prasad R, Adhikari S, Higgins BT. Leaching and anaerobic digestion of poultry litter for biogas production and nutrient transformation. *Waste Management*. 2019;84:413-422. doi:10.1016/j.wasman.2018.11.024
226. Wemheuer F, Taylor JA, Daniel R, et al. Tax4Fun2: prediction of habitat-specific functional profiles and functional redundancy based on 16S rRNA gene sequences. *Environ Microbiome*. 2020;15(1):11. doi:10.1186/s40793-020-00358-7
227. Higgins BT, Gennity I, Fitzgerald PS, Ceballos SJ, Fiehn O, VanderGheynst JS. Algal–bacterial synergy in treatment of winery wastewater. *NPJ Clean Water*. 2018;1(1). doi:10.1038/s41545-018-0005-y
228. Wang Q, Higgins BT, Ji H, Zhao D. Use of photocatalytic nanomaterials for volatile fatty acid removal from anaerobic digestate leads to improved algal growth. In: *2019 Boston, Massachusetts July 7- July 10, 2019*. American Society of Agricultural and Biological Engineers; 2019. doi:10.13031/aim.201900653
229. Sprague D. *Solids Retention Governs Nitrification of Poultry Processing Wastewater Using an Algal-Bacterial Consortium*. Auburn University; 2021.
230. Le TS, Bui XT, Thong PMD, et al. Influence of nitrogen loading rate on nutrient removal and algal biomass production using revolving algae biofilm reactor. *J Taiwan Inst Chem Eng*. 2025;166:105417. doi:10.1016/j.jtice.2024.105417
231. Phanwilai S, Noophan P, Li CW, Choo KH. Effect of COD/N ratio on biological nitrogen removal using full-scale step-feed in municipal wastewater treatment plants. *Sustainable Environment Research*. 2020;30(1):24. doi:10.1186/s42834-020-00064-6
232. Peng Z, Lei Y, Zhan Y, Yang B, Pan X. Impact of Chemical Oxygen Demand/Total Nitrogen Ratio on Shifting Autotrophic Partial Nitrification to Heterotrophic Nitrification and Aerobic Denitrification in High-Strength Ammonium Wastewater Treatment. *Water (Basel)*. 2024;16(17):2532. doi:10.3390/w16172532
233. Khumalo SM, Bakare BF, Rathilal S, Tetteh EK. Characterization of South African Brewery Wastewater: Oxidation-Reduction Potential Variation. *Water (Basel)*. 2022;14(10):1604. doi:10.3390/w14101604
234. Smriga S, Ciccarese D, Babbin AR. Denitrifying bacteria respond to and shape microscale gradients within particulate matrices. *Commun Biol*. 2021;4(1):570. doi:10.1038/s42003-021-02102-4

235. Moser-Engeler R, Udert KM, Wild D, Siegrist H. Products from primary sludge fermentation and their suitability for nutrient removal. *Water Science and Technology*. 1998;38(1):265-273. doi:10.1016/S0273-1223(98)00411-9
236. Zhang J, Peng Y, Li X, Du R. Feasibility of partial-denitrification/ anammox for pharmaceutical wastewater treatment in a hybrid biofilm reactor. *Water Res*. 2022;208:117856. doi:10.1016/j.watres.2021.117856
237. Cheng S, Huai J, Zhong F, Wu J, Yu S. Enhancing denitrification in constructed wetland with algae addition. *Environmental Science and Pollution Research*. 2022;29(2):1949-1960. doi:10.1007/s11356-021-15755-9
238. Phaup JD. The biology of Sphaerotilus species. *Water Res*. 1968;2(9):597-614. doi:10.1016/0043-1354(68)90065-1
239. Curtis EJC. Sewage fungus: Its nature and effects. *Water Res*. 1969;3(5):289-311. doi:10.1016/0043-1354(69)90084-0
240. Eikelboom DH. Filamentous organisms observed in activated sludge. *Water Res*. 1975;9(4):365-388. doi:10.1016/0043-1354(75)90182-7
241. Huang TL, Zhou SL, Zhang HH, Bai SY, He XX, Yang X. Nitrogen Removal Characteristics of a Newly Isolated Indigenous Aerobic Denitrifier from Oligotrophic Drinking Water Reservoir, Zoogloea sp. N299. *Int J Mol Sci*. 2015;16(5):10038-10060. doi:10.3390/ijms160510038
242. Thomsen TR, Kong Y, Nielsen PH. Ecophysiology of abundant denitrifying bacteria in activated sludge. *FEMS Microbiol Ecol*. 2007;60(3):370-382. doi:10.1111/j.1574-6941.2007.00309.x
243. Chipperfield M. Nitrous oxide delays ozone recovery. *Nat Geosci*. 2009;2(11):742-743. doi:10.1038/ngeo678
244. Humpage A, Falconer I, Bernard C, Froscio S, Fabbro L. Toxicity of the cyanobacterium Limnothrix AC0243 to male Balb/c mice. *Water Res*. 2012;46(5):1576-1583. doi:10.1016/j.watres.2011.11.019
245. Daniels O, Fabbro L, Makiela S. The Effects of the Toxic Cyanobacterium Limnothrix (Strain AC0243) on Bufo marinus Larvae. *Toxins (Basel)*. 2014;6(3):1021-1035. doi:10.3390/toxins6031021
246. Rotthauwe JH, Witzel KP, Liesack W. The ammonia monooxygenase structural gene amoA as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. *Appl Environ Microbiol*. 1997;63(12):4704-4712. doi:10.1128/aem.63.12.4704-4712.1997
247. Pester M, Maixner F, Berry D, et al. NxrB encoding the beta subunit of nitrite oxidoreductase as functional and phylogenetic marker for nitrite-oxidizing Nitrospira. *Environ Microbiol*. 2014;16(10):3055-3071. doi:10.1111/1462-2920.12300
248. Henry S, Baudoin E, López-Gutiérrez JC, Martin-Laurent F, Brauman A, Philippot L. Quantification of denitrifying bacteria in soils by nirK gene targeted real-time PCR. *J Microbiol Methods*. 2004;59(3):327-335. doi:10.1016/j.mimet.2004.07.002
249. Chen R, Deng M, He X, Hou J. Enhancing Nitrate Removal from Freshwater Pond by Regulating Carbon/Nitrogen Ratio. *Front Microbiol*. 2017;8. doi:10.3389/fmicb.2017.01712
250. Throbäck IN, Enwall K, Jarvis Ãs, Hallin S. Reassessing PCR primers targeting nirS, nirK and nosZ genes for community surveys of denitrifying bacteria with DGGE. *FEMS Microbiol Ecol*. 2004;49(3):401-417. doi:10.1016/j.femsec.2004.04.011
251. Delorme S, Philippot L, Edel-Hermann V, Deulvot C, Mougel C, Lemanceau P. Comparative Genetic Diversity of the narG, nosZ, and 16S rRNA Genes in Fluorescent Pseudomonads. *Appl Environ Microbiol*. 2003;69(2):1004-1012. doi:10.1128/AEM.69.2.1004-1012.2003

Appendix

Figure A1: Photo of wastewater treatment system and hydroponic setup.

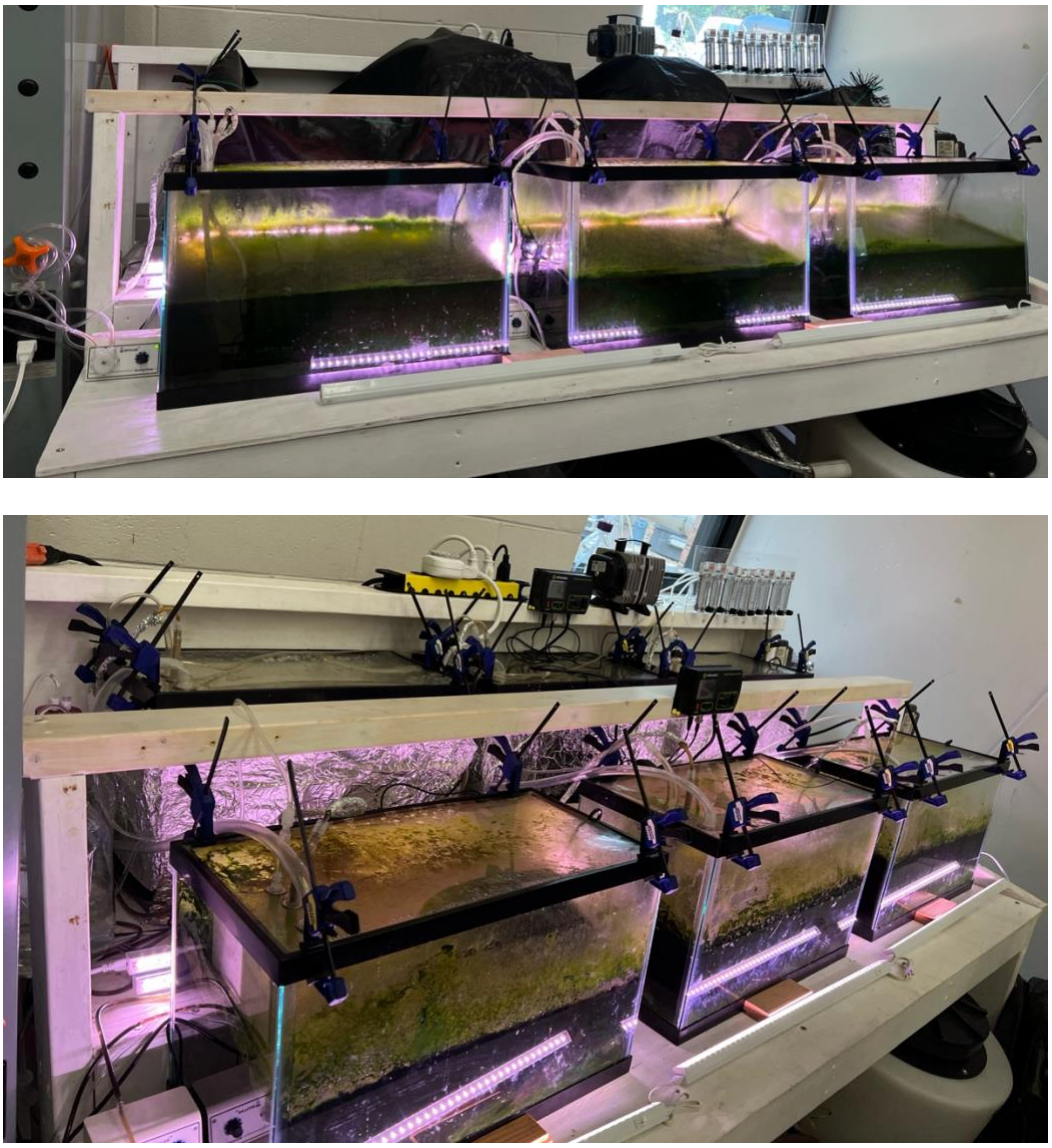


Figure A2: Photo of bioreactors. Algal bioreactors were exposed to light and maintained a dark green color. The bacteria reactors were colored with aluminum foil and a dark cloth to minimize algae growth.

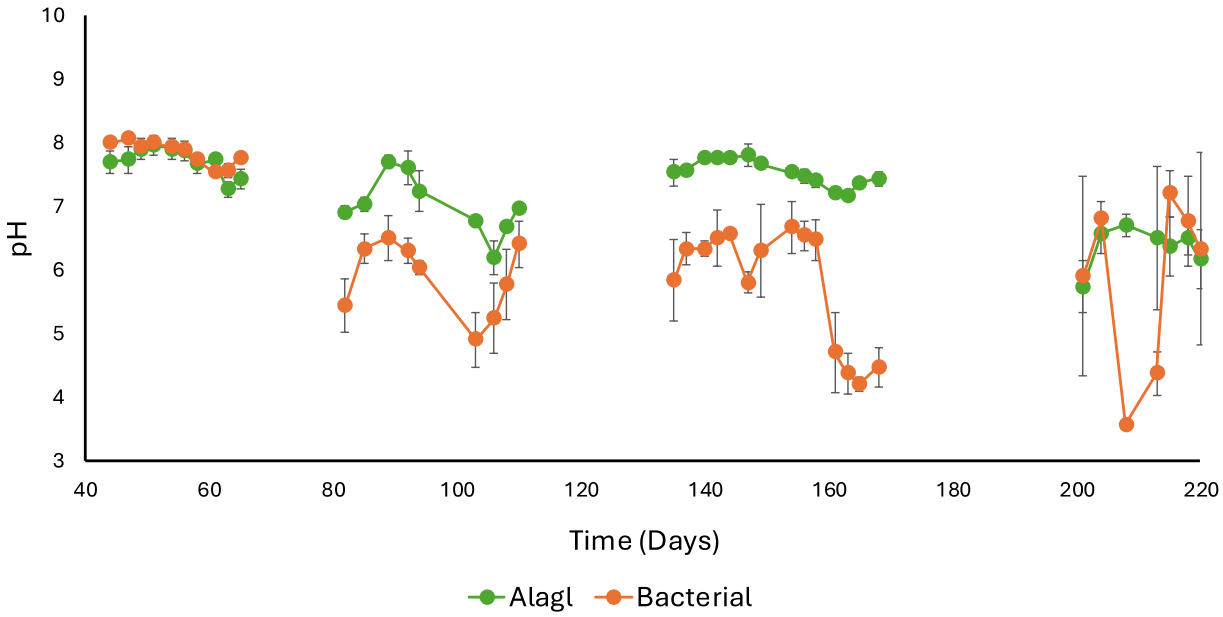


Figure A3: pH measurements in grow beds across experiments.

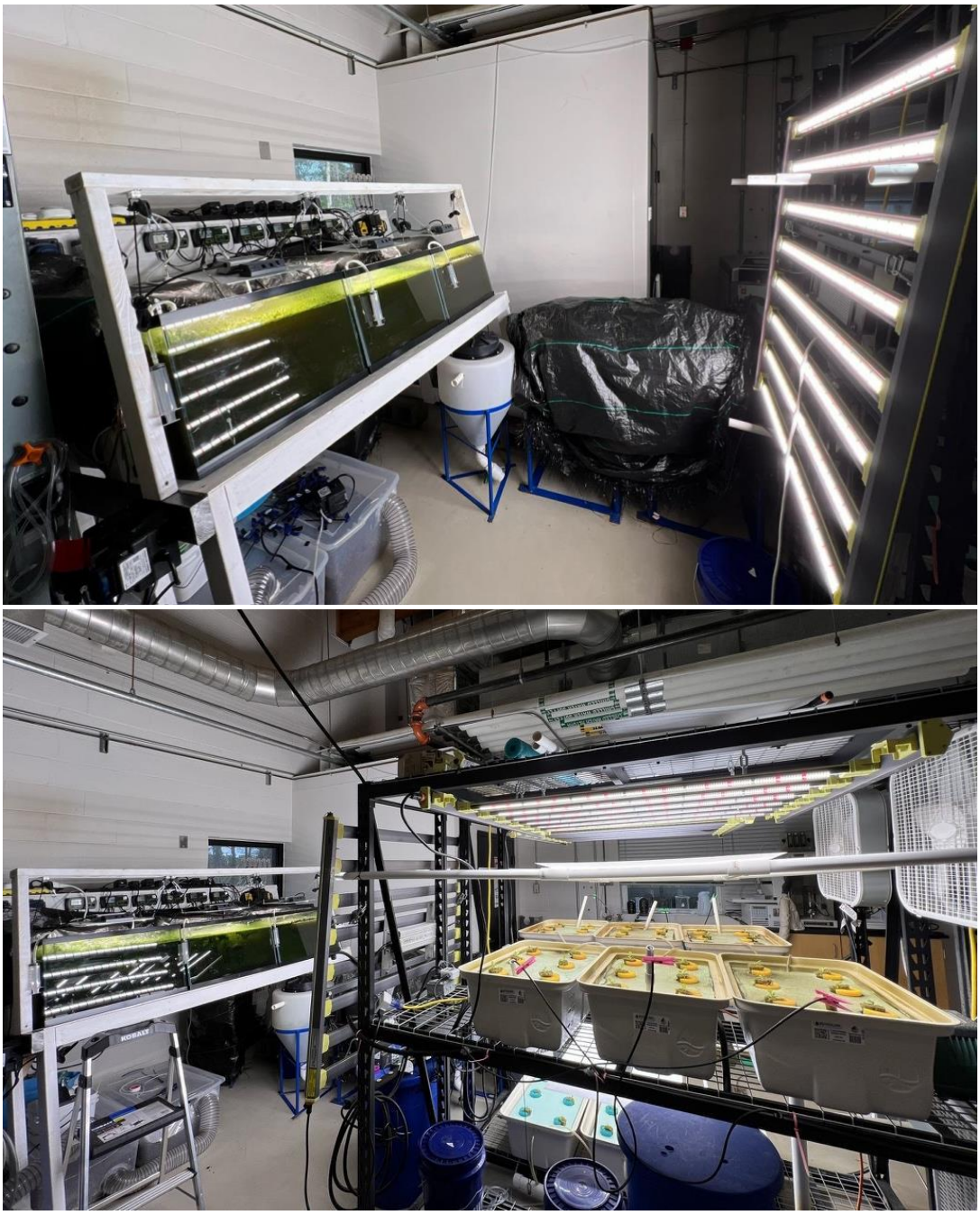


Figure A4: Photos of wastewater treatment system (series setup) and hydroponic setup. Algal bioreactors were exposed to light and maintained a dark green color. The bacteria reactors were colored with aluminum foil and a dark cloth to minimize algae growth.

Table A1: Wastewater characteristics (mg L^{-1}) for each batch used in the experiments

Experiment 1										
Batch	NH_4-N	NO_2-N	NO_3-N	TN	PO_4-P	TP	SO_4-S	K	Na	Cl
1	4.3	0.1	1.3	90	8.5	42.9	6.2	18.7	136.7	70.0
2	7.2	0.1	1.0	75	8.9	48.6	6.3	19.0	141.3	89.9
3	14.0	0.2	1.1	120	7.1	45.1	7.3	21.8	169.2	91.7
4	9.12	0.3	0.9	90	10.5	46.4	6.9	20.8	164.0	94.5
5	12.7	0	1.2	95	9.9	47	11.6	34.7	246.9	137.4
6	8.0	0	1.0	100	9.2	45.5	6.1	18.4	146.4	96.6
Experiment 2										
Batch	NH_4-N	NO_2-N	NO_3-N	TN	PO_4-P	TP	SO_4-S	K	Na	Cl
1	4.3	0.1	0.6	35	11.3	21.5	8.2	59.7	156.5	101.0
2	30.5	0	0.8	60	12.3	37.5	7.9	78.7	191.0	96.8
3	39.0	0	0	35	10.3	24	6.2	41.5	149.9	59.6
4	69.1	0	0	70	18.5	40	12.7	89.5	241.9	109.4
5	8.8	0	0	50	12.6	24	15.2	63.8	192.8	88.6
Experiment 3										
Batch	NH_4-N	NO_2-N	NO_3-N	TN	PO_4-P	TP	SO_4-S	K	Na	Cl
1	29.5	0	0	40	11.6	33	9.5	58.8	169.5	83.0
2	50.6	0	0	60	10.6	21	4.9	71.8	178.4	88.5
3	5.9	0	0.1	30	8.7	32	10.2	58.8	179.3	98.1
4	32.7	0	0.1	35	7.3	28.5	11.6	54.1	142.0	75.3
5	4.5	0	0	55	15.2	37	11.8	44.6	134.3	114.7
Experiment 4										
Batch	NH_4-N	NO_2-N	NO_3-N	TN	PO_4-P	TP	SO_4-S	K	Na	Cl
1	4.5	0	0.1	35	8.6	37	11.4	57.6	127.9	84.0
2	74.1	0	0.4	117	13.5	50.5	12.0	84.8	201.8	114.4
3	63.9	0	0.5	81	10.9	40.5	8.0	74.6	165.6	77.6
4	52.4	0	0	55	10.6	46.5	14.0	71.1	185.9	94.1
5	45.4	0	0.2	40	9.3	39	9.0	50.6	113.3	82.9

Table A2: Average concentration of non-N nutrients entering (influent) and exiting (effluent) the lettuce grow beds in each experiment compared to the recommended non-N nutrient concentrations in lettuce hydroponic solution (Sonneveld 1994).

Parameters	Influent							
	Algal system				Bacterial system			
	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Experiment 1	Experiment 2	Experiment 3	Experiment 4
PO_4-P (mg L^{-1})	12.0 ± 2.1 [a]	12.2 ± 2.2 [a]	13.4 ± 2.8 [a]	11.7 ± 2.4 [a]	12.1 ± 2.4 [a]	13.6 ± 2.6 [a]	13.5 ± 2.7 [a]	12.2 ± 5.5 [a]
SO_4-S (mg L^{-1})	12.6 ± 2.3 [c]	34.1 ± 27.3 [c]	19.1 ± 6.6 [c]	51.8 ± 33.5 [b]	13.1 ± 2.6 [c]	160.1 ± 71.7 [a]	172.3 ± 50.4 [a]	79.5 ± 51.9 [b]
K^+ (mg L^{-1})	80.2 ± 11.6 [a]	63.6 ± 19.3 [b]	72.6 ± 20.5 [ab]	65.1 ± 16.8 [b]	84.6 ± 13.7 [a]	65.6 ± 17.6 [b]	72.3 ± 16.9 [ab]	64.3 ± 16.8 [b]
Na^+ (mg L^{-1})	176.7 ± 30.4 [ab]	176.5 ± 43.9 [ab]	204.2 ± 53.2 [a]	149.4 ± 39.7 [b]	185.9 ± 30.2 [ab]	201.4 ± 44.3 [a]	225.0 ± 59.8 [a]	155.9 ± 53.5 [b]
Cl^- (mg L^{-1})	102.9 ± 11.5 [a]	98.2 ± 17.4 [a]	98.3 ± 23.4 [a]	82.2 ± 17.4 [b]	104.8 ± 13.3 [a]	98.8 ± 20.2 [a]	92.5 ± 16.6 [b]	80.6 ± 11.9 [b]
pH	7.5 ± 0.2 [a]	7.3 ± 0.2 [a]	7.4 ± 0.1 [a]	7.1 ± 0.1 [ab]	7.8 ± 0.1 [a]	6.7 ± 0.6 [b]	6.8 ± 0.1 [b]	7.1 ± 0.1 [ab]

Parameters	Effluent								Hydroponic solution [73]
	Algal system				Bacterial system				
	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Experiment 1	Experiment 2	Experiment 3	Experiment 4	
PO_4-P (mg L^{-1})	11.4 ± 2.0 [b]	12.2 ± 3.5 [b]	13.0 ± 2.0 [b]	13.0 ± 3.3 [b]	12.8 ± 2.7 [b]	13.5 ± 3.3 [a]	13.6 ± 3.0 [b]	12.7 ± 3.2 [b]	62
SO_4-S (mg L^{-1})	12.6 ± 2.2 [c]	35.5 ± 17.8 [c]	21.3 ± 4.9 [c]	49.0 ± 30.9 [b]	15.0 ± 2.7 [c]	181.7 ± 35.1 [a]	184.7 ± 47.0 [a]	79.0 ± 40.2 [b]	36.1
K^+ (mg L^{-1})	78.0 ± 10.6 [a]	57.5 ± 26.3 [b]	65.7 ± 13.5 [ab]	68.2 ± 19.8 [ab]	85.6 ± 23.1 [a]	58.7 ± 18.3 [b]	68.2 ± 17.4 [ab]	65.5 ± 17.8 [ab]	430.1
Na^+ (mg L^{-1})	183.5 ± 25.9 [ab]	196.5 ± 45.8 [ab]	214.8 ± 43.4 [a]	168.3 ± 41.4 [b]	197.9 ± 31.7 [ab]	212.5 ± 37.4 [a]	230.3 ± 47.2 [a]	164.2 ± 37.3 [b]	-
Cl^- (mg L^{-1})	106.4 ± 15.4 [a]	94.6 ± 20.7 [b]	103.8 ± 18.5 [a]	90.9 ± 16.1 [b]	113.1 ± 19.4 [a]	89.7 ± 15.9 [b]	94.1 ± 15.3 [b]	88.4 ± 13.6 [b]	-
pH	7.7 ± 0.2 [a]	7.0 ± 0.5 [ab]	7.5 ± 0.2 [a]	6.3 ± 0.5 [b]	7.8 ± 0.2 [a]	5.9 ± 0.6 [b]	5.8 ± 0.9 [b]	6.0 ± 1.3 [b]	5.5 – 6.5

For each non-N nutrient, means with the same letter are not statistically different.

Table A3: Average concentration of non-N nutrients in in bioreactors in each experiment compared to the recommended non-N nutrient concentrations in lettuce hydroponic solution (Sonneveld 1994) [73].

Average concentration (mg/L)	Grow beds								Hydroponic solution [73]
	Algal system				Bacterial system				
	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Experiment 1	Experiment 2	Experiment 3	Experiment 4	
PO_4-P	12.5 ± 5.1	13.9 ± 4.3	11.9 ± 1.8	11.8 ± 1.7	11.7 ± 4.3	14.1 ± 3.8	12.8 ± 1.7	11.3 ± 1.7	62
SO_4-S	11.0 ± 5.0	31.7 ± 14.9	17.4 ± 3.8	55.8 ± 35.5	11.2 ± 4.1	147.5 ± 66.5	169.8 ± 52.9	80.7 ± 43.5	36.1
K	32.9 ± 15.1	63.1 ± 11.5	67.8 ± 11.0	66.9 ± 13.1	33.6 ± 12.2	57.8 ± 17.7	70.3 ± 11.8	67.9 ± 11.7	430.1
Na	191.7 ± 39.6	177.5 ± 24.8	193.4 ± 30.1	161.1 ± 31.6	180.0 ± 28.7	175.5 ± 30.8	208.1 ± 33.7	164.4 ± 31.6	-
Cl	108.5 ± 22.5	96.4 ± 15.2	94.0 ± 10.7	86.5 ± 10.8	103.3 ± 18.8	92.7 ± 17.7	93.9 ± 14.3	86.4 ± 18.7	-
pH	7.3 ± 0.2	6.8 ± 0.1	6.8 ± 0.1	6.7 ± 0.2	7.7 ± 0.5	6.8 ± 0.2	6.9 ± 0.1	6.5 ± 0.2	5.5 – 6.5

Table A4: Composition of poultry processing wastewater (mg L^{-1}) used in Phase I, II and III

Phase I (n = 6)												
sCOD	VFA	TN	NH_4-N	NO_2-N	NO_3-N	TP	PO_4-P	SO_4-S	K^+	Na^+	Cl^-	pH
481 ± 109	391 ± 157	95 ± 15	9 ± 4	0.1 ± 0.1	1.1 ± 0.1	46 ± 2	9 ± 1	7 ± 2	22 ± 6	167 ± 41	97 ± 22	7.2 ± 0.1
Phase II (n = 10)												
sCOD	VFA	TN	NH_4-N	NO_2-N	NO_3-N	TP	PO_4-P	SO_4-S	K^+	Na^+	Cl^-	pH
558 ± 177	243 ± 72	48 ± 14	27 ± 22	0.0 ± 0.0	0.3 ± 0.2	30 ± 7	12 ± 3	10 ± 3	62 ± 15	173 ± 31	92 ± 16	6.9 ± 0.4
Phase III (n=5)												
sCOD	VFA	TN	NH_4-N	NO_2-N	NO_3-N	TP	PO_4-P	SO_4-S	K^+	Na^+	Cl^-	pH
667 ± 173	358 ± 39	48 ± 12	48 ± 27	0.0 ± 0.0	0.2 ± 0.1	43 ± 6	11 ± 2	11 ± 2	68 ± 14	159 ± 38	91 ± 15	7.0 ± 0.2

Values are means ± standard deviation of the batches of poultry processing wastewater collected for each phase

Table A5: Similarity percentage breakdown analysis for top 20 genera of prokaryotes in bioreactors at the start of the experiment and end of Phase I.

Algal bioreactors					Bacterial bioreactors				
Genus	Average dissimilarity	Contribution (%)	Mean abundance (%) Starting culture	Mean abundance (%) Phase I	Genus	Average dissimilarity	Contribution (%)	Mean abundance (%) Starting culture	Mean abundance (%) Phase I
acinetobacter	7.8	12.9	15.9	0.3	*brachymonas*	8.4	12.7	1.0	17.7
comamonas	4.4	7.3	13.5	4.8	*acinetobacter*	8.2	12.4	16.7	0.3
rhodocyclus	4.1	6.8	0.8	9.0	*comamonas*	7.7	11.7	19.6	4.1
luteimonas	3.8	6.2	8.9	2.3	*terrimonas*	2.0	3.0	1.5	5.2
desulfomicrobium	2.3	3.7	0.4	4.9	*sediminibacterium*	1.8	2.8	5.3	1.7
pseudomonas	1.9	3.1	4.4	0.7	*lewinella*	1.4	2.2	2.7	3.4
chryseobacterium	1.7	2.8	3.5	0.1	*thermomonas*	1.4	2.1	3.2	0.5
cloacibacterium	1.5	2.5	0.1	3.1	*aquaspirillum*	1.3	1.9	1.9	2.3
zoogloea	1.3	2.1	5.5	3.3	*longilinea*	1.1	1.6	0.1	2.2
solitalea	1.3	2.1	0.1	2.7	*arcobacter*	1.1	1.6	0.0	2.2
sediminibacterium	1.1	1.8	3.9	1.8	*acidovorax*	1.0	1.5	3.0	1.0
pontibacter	0.9	1.6	0.0	1.9	*alicycliphilus*	0.9	1.3	2.8	1.1
thauera	0.7	1.2	0.2	1.7	*chryseobacterium*	0.9	1.3	1.8	0.0
thermomonas	0.7	1.2	1.9	0.4	*sulfuritalea*	0.9	1.3	0.2	1.8
paludibacter	0.7	1.2	0.1	1.5	*thauera*	0.8	1.2	0.5	1.9
ornatilinea	0.7	1.1	0.2	1.6	*ornatilinea*	0.8	1.2	0.2	1.7
acidovorax	0.7	1.1	1.6	2.9	*desulfomicrobium*	0.8	1.2	0.4	1.9
longilinea	0.6	1.0	0.1	1.4	*rhodocyclus*	0.7	1.1	0.5	2.0
malikia	0.6	1.0	0.0	1.2	*pseudomonas*	0.7	1.1	1.8	0.3
hydrogenophaga	0.6	1.0	0.1	1.3	*delftia*	0.7	1.1	1.5	0.0

Table A6: Similarity percentage breakdown analysis for top 20 genera of prokaryotes in bioreactors between Phase I and Phase 2.

Algal bioreactors					Bacterial bioreactors				
Genus	Average dissimilarity	Contribution (%)	Mean abundance (%) Phase I	Mean abundance (%) Phase II	Genus	Average dissimilarity	Contribution (%)	Mean abundance (%) Phase I	Mean abundance (%) Phase II
phormidium	4.3	7.5	0.0	8.7	*brachymonas*	8.2	11.7	17.7	1.3
rhodocyclus	3.6	6.2	9.0	1.8	*thiomonas*	5.8	8.3	0.0	11.6
longilinea	2.7	4.7	1.4	6.8	*candidatus nardonella*	4.6	6.6	0.0	9.3
ornatilinea	2.1	3.7	1.6	5.8	*lewinella*	3.8	5.4	3.4	9.8
dechloromonas	1.8	3.1	1.3	3.9	*comamonas*	2.5	3.6	4.1	8.6
zoogloea	1.6	2.8	3.3	0.0	*terrimonas*	2.4	3.4	5.2	0.4
desulfomicrobium	1.6	2.7	4.9	1.8	*thermomonas*	1.9	2.7	0.5	4.3
cloacibacterium	1.5	2.6	3.1	0.1	*hydrotalea*	1.4	1.9	0.0	2.7
comamonas	1.5	2.6	4.8	1.9	*pandoraea*	1.3	1.9	0.0	2.6
candidatus competibacter	1.4	2.4	0.0	2.8	*aquaspirillum*	1.1	1.6	2.3	0.0
brachymonas	1.0	1.8	1.0	3.1	*arcobacter*	1.1	1.5	2.2	0.0
rubrivivax	1.0	1.7	2.7	0.8	*longilinea*	1.0	1.5	2.2	0.2
luteimonas	0.9	1.6	2.3	0.5	*rubrivivax*	1.0	1.4	2.1	0.2
pontibacter	0.9	1.6	1.9	0.1	*thauera*	0.9	1.3	1.9	0.1
acidovorax	0.9	1.6	2.9	1.1	*sulfuritalea*	0.9	1.3	1.8	0.0
sediminibacterium	0.8	1.4	1.8	0.1	*desulfomicrobium*	0.9	1.3	1.9	0.2
clostridium	0.8	1.4	0.7	2.3	*sediminibacterium*	0.8	1.2	1.7	0.1
thauera	0.8	1.4	1.7	0.1	*dechloromonas*	0.8	1.1	1.2	2.1
ignavibacterium	0.7	1.3	0.2	1.7	*rhodocyclus*	0.8	1.1	2.0	2.6
paludibacter	0.6	1.1	1.5	0.2	*leucobacter*	0.7	1.1	0.6	2.1

Table A7: Similarity percentage breakdown analysis for top 20 genera of prokaryotes in bioreactors between Phase 2 and Phase 3.

Algal bioreactors					Bacterial bioreactors				
Genus	Average dissimilarity	Contribution (%)	Mean abundance (%) Phase II	Mean abundance (%) Phase III	Genus	Average dissimilarity	Contribution (%)	Mean abundance (%) Phase II	Mean abundance (%) Phase III
phormidium	3.8	6.9	8.7	1.5	*thiomonas*	3.9	7.5	11.6	3.7
rhodocyclus	3.3	6.0	1.8	8.5	*candidatus nardonella*	3.5	6.7	9.3	2.3
longilinea	2.4	4.2	6.8	2.1	*lewinella*	3.3	6.3	9.8	5.0
kamptonema	2.2	3.9	1.1	4.0	*methylotenera*	3.3	6.2	0.0	6.6
limnothrix	1.9	3.4	0.0	3.8	*comamonas*	2.6	5.0	8.6	3.8
dechloromonas	1.9	3.4	3.9	2.0	*brachymonas*	2.5	4.8	1.3	6.4
chlorobaculum	1.7	3.1	0.8	3.9	*rhodocyclus*	2.1	3.9	2.6	6.6
ornatilinea	1.7	3.0	5.8	2.5	*desulfatirhabdium*	1.3	2.5	0.2	2.8
candidatus nitrotoga	1.5	2.6	0.2	3.1	*thermomonas*	1.2	2.3	4.3	2.1
methylotenera	1.3	2.3	0.0	2.6	*hydrotalea*	1.0	2.0	2.7	0.7
chlorobium	1.3	2.3	0.1	2.6	*aquabacter*	1.0	1.8	0.2	2.0
sulfuricurvum	1.1	2.0	0.0	2.3	*geobacter*	0.9	1.7	0.0	1.8
brachymonas	1.0	1.8	3.1	2.7	*spirochaeta*	0.8	1.5	0.4	2.0
candidatus competibacter	0.9	1.7	2.8	0.9	*dechloromonas*	0.8	1.5	2.1	2.9
zoogloea	0.8	1.4	0.0	1.6	*pandoraea*	0.7	1.4	2.6	1.1
desulfonema	0.8	1.4	0.0	1.5	*holophaga*	0.7	1.3	0.4	1.8
thauera	0.6	1.1	0.1	1.4	*desulfocapsa*	0.6	1.2	1.6	0.7
comamonas	0.6	1.1	1.9	1.9	*ignavibacterium*	0.6	1.2	0.2	1.4
desulfomicrobium	0.6	1.0	1.8	0.9	*leucobacter*	0.6	1.1	2.1	1.1
clostridium	0.5	1.0	2.3	1.2	*dokdonella*	0.5	1.0	1.0	1.1

Table A8: Similarity percentage breakdown analysis for top 20 genera of prokaryotes in hydroponic reservoirs between Phase I and Phase 2.

Algal grow beds					Bacterial grow beds				
Genus	Average dissimilarity	Contribution (%)	Mean abundance (%) Phase I	Mean abundance (%) Phase II	Genus	Average dissimilarity	Contribution (%)	Mean abundance (%) Phase I	Mean abundance (%) Phase II
comamonas	9.7	12.2	19.6	0.1	*rudaea*	6.8	8.5	0.0	13.7
kamptonema	8.5	10.6	0.0	17.0	*flavisolibacter*	5.2	6.5	0.0	10.5
candidatus protistobacter	7.7	9.7	0.0	15.5	*candidatus rhabdochlamydia*	4.8	6.0	9.8	0.1
acidovorax	3.9	4.8	7.9	0.2	*haliscomenobacter*	3.3	4.1	6.6	0.1
pseudomonas	3.3	4.1	6.7	0.1	*neochlamydia*	2.7	3.4	0.3	5.6
runella	2.4	3.0	5.1	0.4	*azospirillum*	2.4	3.0	0.0	4.8
aridibacter	2.4	3.0	0.0	4.8	*comamonas*	2.2	2.7	4.6	0.3
wilmottia	2.0	2.4	0.0	3.9	*zavarzinella*	1.8	2.3	0.5	4.1
candidatus babela	1.6	2.0	0.3	3.5	*alkaliflexus*	1.8	2.2	3.8	0.2
candidatus metachlamydia	1.4	1.7	4.1	1.3	*rhodanobacter*	1.7	2.1	0.0	3.3
leptolyngbya	1.0	1.3	0.0	2.1	*pseudoxanthomonas*	1.4	1.7	2.7	0.0
erythrobacter	0.8	1.0	1.6	0.0	*thauera*	1.4	1.7	2.7	0.0
ignavibacterium	0.8	1.0	1.9	0.7	*lacibacter*	1.2	1.5	0.2	2.6
dokdonella	0.7	0.9	1.6	0.2	*hirschia*	1.1	1.4	2.3	0.0
zoogloea	0.6	0.8	1.3	0.0	*gemmata*	1.1	1.4	0.4	2.7
candidatus anammoximicrobium	0.6	0.8	0.0	1.3	*dokdonella*	1.0	1.3	0.9	2.4
sphingobacterium	0.6	0.8	1.2	0.0	*gemmatimonas*	0.9	1.1	2.2	2.6
niastella	0.6	0.8	0.0	1.2	*terrimonas*	0.9	1.1	2.6	1.4
shinella	0.6	0.8	1.3	0.1	*hydrogenophaga*	0.9	1.1	1.8	0.0
pseudoxanthomonas	0.6	0.7	1.2	0.0	*runella*	0.9	1.1	1.8	0.0

Table A9: Similarity percentage breakdown analysis for top 20 genera of prokaryotes in hydroponic reservoirs between Phase 2 and Phase 3.

Algal grow beds					Bacterial grow beds				
Genus	Average dissimilarity	Contribution (%)	Mean abundance (%) Phase II	Mean abundance (%) Phase III	Genus	Average dissimilarity	Contribution (%)	Mean abundance (%) Phase II	Mean abundance (%) Phase III
thermomonas	8.7	11.3	0.0	17.4	*dokdonella*	5.8	9.3	2.4	14.0
candidatus protistobacter	7.7	10.0	15.5	0.1	*rudaea*	5.4	8.6	13.7	2.9
kamptonema	7.5	9.7	17.0	2.1	*flavisolibacter*	4.6	7.3	10.5	1.4
rudaea	2.5	3.3	0.1	5.2	*neochlamydia*	2.8	4.5	5.6	0.2
aridibacter	2.4	3.1	4.8	0.0	*rhodanobacter*	2.6	4.1	3.3	7.5
chitinophaga	2.1	2.8	0.0	4.3	*rhodocyclus*	2.5	4.0	0.8	5.7
wilmottia	2.0	2.5	3.9	0.0	*aquabacter*	2.4	3.8	0.0	4.8
lacibacter	1.7	2.2	0.0	3.4	*azospirillum*	2.4	3.8	4.8	0.0
sphingomonas	1.5	2.0	0.5	3.5	*pontibacter*	2.3	3.7	1.8	6.4
spirosoma	1.4	1.8	0.0	2.8	*zavarzinella*	2.1	3.3	4.1	0.0
candidatus babela	1.3	1.7	3.5	2.6	*nitrosovibrio*	1.4	2.2	1.8	4.3
dokdonella	1.2	1.6	0.2	2.6	*burkholderia*	1.2	1.9	0.6	2.8
pontibacter	1.2	1.5	0.0	2.3	*gemmatimonas*	1.1	1.8	2.6	0.3
leptolyngbya	1.0	1.3	2.1	0.0	*solitalea*	1.0	1.6	0.3	2.2
wandonia	0.9	1.2	0.0	1.9	*gemmata*	0.9	1.4	2.7	0.9
lewinella	0.9	1.2	0.2	2.0	*lysobacter*	0.8	1.3	0.3	1.9
erythrobacter	0.8	1.1	0.0	1.7	*lacibacter*	0.8	1.3	2.6	4.2
rhizomicrobium	0.7	0.9	0.1	1.5	*reyranella*	0.7	1.1	1.4	1.7
chryseobacterium	0.7	0.9	0.1	1.4	*thermomonas*	0.7	1.1	1.9	1.2
solitalea	0.7	0.9	0.0	1.4	*wandonia*	0.7	1.1	0.9	2.1

Table A10: Similarity percentage breakdown analysis for top 20 genera of eukaryotes in bioreactors at the start of the experiment and end of Phase I.

Algal bioreactors					Bacterial bioreactors				
Genus	Average dissimilarity	Contribution (%)	Mean abundance (%) Starting culture	Mean abundance (%) Phase I	Genus	Average dissimilarity	Contribution (%)	Mean abundance (%) Starting culture	Mean abundance (%) Phase I
adineta	12.6	30.1	40.2	51.8	*adineta*	21.1	30.8	23.7	52.2
clavispora	4.2	10.1	12.0	3.5	*vorticellides*	13.1	19.1	1.4	26.9
ephelota	4.2	9.9	0.1	8.4	*clavispora*	10.6	15.5	26.9	5.8
desmodesmus	4.2	9.9	17.7	9.4	*clavulina*	7.5	11.0	18.7	3.6
vorticellides	2.8	6.7	0.0	5.7	*desmodesmus*	3.7	5.3	8.7	1.4
clavulina	2.3	5.5	8.8	4.7	*rhogostoma*	3.5	5.1	7.1	0.1
rhogostoma	1.9	4.6	4.4	0.6	*kockovaella*	1.0	1.5	2.8	0.7
neochloris	1.6	3.8	0.8	3.9	*steinia*	1.0	1.4	0.2	2.1
chlamydomonas	1.0	2.3	2.2	0.3	*epiphanes*	0.7	1.1	1.7	0.2
aeolosoma	0.9	2.2	1.8	0.0	*paramecium*	0.6	0.9	1.2	0.0
parachlorella	0.8	1.9	3.9	2.7	*chaetogaster*	0.6	0.8	0.0	1.1
paramecium	0.5	1.1	1.0	0.0	*rhizopus*	0.5	0.7	1.4	0.5
euglena	0.4	1.0	0.0	0.9	*euplotes*	0.4	0.6	0.0	0.8
acrasis	0.4	0.9	0.0	0.7	*naegleria*	0.3	0.5	0.6	0.0
characium	0.4	0.9	0.7	0.8	*oscheius*	0.3	0.5	0.5	1.0
kockovaella	0.3	0.8	1.2	0.5	*rozella*	0.3	0.4	0.0	0.6
rozella	0.3	0.7	0.4	0.9	*acrorhynchides*	0.3	0.4	0.6	0.0
katablepharis	0.2	0.5	0.0	0.5	*sporobolomyces*	0.3	0.4	0.8	0.2
rhizopus	0.2	0.5	0.7	0.4	*hydractinia*	0.2	0.3	0.6	0.1
umbelopsis	0.2	0.5	0.0	0.4	*apodileptus*	0.2	0.3	0.0	0.4

Table A11: Similarity percentage breakdown analysis for top 20 genera of eukaryotes in bioreactors between Phase I and Phase 2.

Algal bioreactors					Bacterial bioreactors				
Genus	Average dissimilarity	Contribution (%)	Mean abundance (%) Phase I	Mean abundance (%) Phase II	Genus	Average dissimilarity	Contribution (%)	Mean abundance (%) Phase I	Mean abundance (%) Phase II
adineta	25.1	34.3	51.8	1.7	*adineta*	15.6	33.6	52.2	69.5
desmodesmus	18.2	24.9	9.4	45.8	*vorticellides*	13.1	28.3	26.9	1.1
neochloris	6.8	9.3	3.9	17.4	*candida*	2.4	5.1	0.0	4.8
mucor	4.1	5.6	0.0	8.2	*clavispora*	2.4	5.1	5.8	5.7
ephelota	3.7	5.1	8.4	3.0	*umbelopsis*	2.3	5.0	0.0	4.7
vorticellides	2.5	3.4	5.7	0.8	*clavulina*	1.4	2.9	3.6	1.5
rozella	1.7	2.3	0.9	4.2	*steinia*	1.0	2.1	2.1	0.3
clavispora	1.5	2.0	3.5	4.9	*mesodinium*	0.9	1.8	0.1	1.8
clavulina	1.4	2.0	4.7	3.1	*apodileptus*	0.7	1.4	0.4	1.2
parachlorella	0.9	1.2	2.7	1.5	*chaetogaster*	0.6	1.2	1.1	0.0
coelastrum	0.6	0.8	0.1	1.3	*oscheius*	0.5	1.1	1.0	0.0
paramecium	0.6	0.8	0.0	1.2	*desmodesmus*	0.5	1.1	1.4	0.6
sterigmatomyces	0.6	0.8	0.0	1.1	*rhizopus*	0.5	1.0	0.5	1.2
euglena	0.4	0.6	0.9	0.0	*euplotes*	0.4	0.9	0.8	0.0
characium	0.3	0.5	0.8	0.1	*trichosporon*	0.4	0.8	0.0	0.8
acrasis	0.3	0.4	0.7	0.2	*naegleria*	0.4	0.8	0.0	0.7
rhogostoma	0.3	0.4	0.6	0.0	*acrasis*	0.3	0.6	0.3	0.7
katablepharis	0.2	0.3	0.5	0.1	*rozella*	0.3	0.6	0.6	0.7
sphaeronaemella	0.2	0.3	0.0	0.4	*kockovaella*	0.3	0.6	0.7	0.3
umbelopsis	0.2	0.3	0.4	0.0	*paramecium*	0.2	0.4	0.0	0.4

Table A12: Similarity percentage breakdown analysis for top 20 genera of eukaryotes in bioreactors between Phase 2 and Phase 3.

Algal bioreactors					Bacterial bioreactors				
Genus	Average dissimilarity	Contribution (%)	Mean abundance (%) Phase II	Mean abundance (%) Phase III	Genus	Average dissimilarity	Contribution (%)	Mean abundance (%) Phase II	Mean abundance (%) Phase III
desmodesmus	12.9	21.0	45.8	20.0	*adineta*	32.0	39.6	69.5	5.5
adineta	8.2	13.4	1.7	15.9	*vorticellides*	15.2	18.8	1.1	31.5
neochloris	6.0	9.7	17.4	5.5	*clevelandella*	13.7	16.9	0.2	27.5
rozella	5.7	9.3	4.2	14.2	*umbelopsis*	2.4	3.0	4.7	0.8
sphaeronaemella	5.0	8.1	0.4	10.0	*candida*	2.2	2.7	4.8	5.5
mucor	4.1	6.6	8.2	0.0	*clavispora*	2.0	2.5	5.7	3.6
clevelandella	2.7	4.5	0.1	5.6	*epalxella*	1.5	1.8	0.0	2.9
clavispora	2.0	3.3	4.9	1.1	*paramecium*	1.3	1.6	0.4	2.9
paramecium	1.9	3.1	1.2	3.8	*mesodinium*	0.9	1.2	1.8	1.3
ephelota	1.6	2.7	3.0	1.2	*katablepharis*	0.9	1.1	0.0	1.8
euglena	1.4	2.2	0.0	2.8	*apodileptus*	0.6	0.7	1.2	0.0
parachlorella	1.2	1.9	1.5	3.4	*entamoeba*	0.6	0.7	0.0	1.1
clavulina	1.0	1.7	3.1	1.2	*euglena*	0.5	0.7	0.0	1.1
tricellaria	1.0	1.6	0.0	2.0	*clavulina*	0.5	0.6	1.5	1.5
epiphanes	0.7	1.2	0.3	1.7	*hyaloraphidium*	0.4	0.5	0.4	1.2
coelastrum	0.7	1.1	1.3	1.7	*rhizopus*	0.4	0.5	1.2	0.7
vorticellides	0.6	1.0	0.8	1.3	*naegleria*	0.4	0.5	0.7	0.6
slavina	0.4	0.7	0.0	0.8	*ephelota*	0.4	0.4	0.3	1.0
chlorella	0.4	0.6	0.2	0.9	*rozella*	0.3	0.4	0.7	0.8
sterigmatomyces	0.4	0.6	1.1	0.4	*tetrahymena*	0.3	0.3	0.0	0.6

Table A13: Similarity percentage breakdown analysis for top 20 genera of eukaryotes in hydroponic reservoirs between Phase I and Phase 2.

Algal grow beds					Bacterial grow beds				
Genus	Average dissimilarity	Contribution (%)	Mean abundance (%) Phase I	Mean abundance (%) Phase II	Genus	Average dissimilarity	Contribution (%)	Mean abundance (%) Phase I	Mean abundance (%) Phase II
adineta	26.0	28.4	51.9	0.0	*rhogostoma*	33.1	35.3	64.6	1.1
rhogostoma	14.1	15.4	30.1	2.0	*parazoanthus*	13.6	14.5	0.0	26.6
trechispora	11.2	12.3	0.0	22.4	*desmodesmus*	11.1	11.9	0.0	21.5
desmodesmus	9.4	10.2	0.4	19.1	*adineta*	5.8	6.1	11.3	0.3
candida	4.7	5.1	7.9	6.0	*vorticellides*	5.1	5.4	10.1	0.4
acrasis	3.5	3.9	0.0	7.1	*candida*	4.2	4.5	0.5	8.7
schizoplasmodium	3.3	3.6	0.0	6.5	*sterigmatomyces*	4.0	4.3	0.0	7.8
sphaeronaemella	1.8	2.0	0.0	3.6	*lepidodermella*	2.7	2.9	0.0	5.0
clavispora	1.5	1.6	0.1	3.0	*steinia*	1.9	2.0	4.9	1.2
rozella	1.2	1.3	0.4	2.8	*parachlorella*	1.2	1.2	0.0	2.3
steinia	1.1	1.2	2.5	1.9	*hydractinia*	1.1	1.2	3.1	1.3
vorticellides	1.1	1.2	3.0	1.6	*dileptus*	1.0	1.0	0.0	1.8
neochloris	1.1	1.2	0.0	2.2	*trechispora*	0.8	0.9	0.0	1.7
apodileptus	1.1	1.2	0.0	2.1	*phlyctochytrium*	0.8	0.8	0.0	1.5
parachlorella	0.8	0.9	0.4	2.0	*hyaloraphidium*	0.7	0.8	0.0	1.4
chrysosporium	0.7	0.8	0.0	1.4	*tricellaria*	0.6	0.6	0.0	1.1
gliophorus	0.7	0.7	0.0	1.3	*euplotes*	0.5	0.5	0.9	0.0
caespitella	0.6	0.7	0.0	1.3	*vannella*	0.3	0.3	0.6	0.0
myxozyma	0.6	0.7	0.0	1.3	*apodileptus*	0.3	0.3	0.0	0.5
coelastrum	0.6	0.6	0.0	1.2	*trichosporon*	0.3	0.3	0.0	0.6

Table A14: Similarity percentage breakdown analysis for top 20 genera of eukaryotes in hydroponic reservoirs between Phase 2 and Phase 3.

Algal grow beds					Bacterial grow beds				
Genus	Average dissimilarity	Contribution (%)	Mean abundance (%) Phase II	Mean abundance (%) Phase III	Genus	Average dissimilarity	Contribution (%)	Mean abundance (%) Phase II	Mean abundance (%) Phase III
parazoanthus	11.4	15.5	0.0	22.8	*adineta*	18.8	25.7	0.3	36.2
trechispora	10.2	13.9	22.4	3.0	*desmodesmus*	8.9	12.2	21.5	4.3
desmodesmus	5.9	8.0	19.1	9.4	*parazoanthus*	7.5	10.3	26.6	14.1
mallomonas	3.8	5.2	0.1	7.8	*mycoemilia*	4.4	6.0	0.0	8.4
adineta	3.6	4.9	0.0	7.2	*candida*	4.1	5.7	8.7	0.7
lepidodermella	3.1	4.2	0.2	6.0	*melosira*	3.9	5.4	0.0	7.6
acrasis	2.9	4.0	7.1	2.9	*sterigmatomyces*	3.9	5.3	7.8	0.3
candida	2.5	3.5	6.0	1.2	*tricellaria*	2.9	4.0	1.1	6.6
schizoplasmodium	2.2	2.9	6.5	3.6	*lepidodermella*	2.4	3.4	5.0	0.5
rhogostoma	2.1	2.9	2.0	5.2	*acrasis*	1.4	1.9	0.4	3.1
sphaeronaemella	1.8	2.5	3.6	0.4	*hydractinia*	1.2	1.7	1.3	3.4
chrysosporium	1.7	2.3	1.4	3.6	*aeolosoma*	1.0	1.4	0.2	2.1
clavispora	1.3	1.8	3.0	0.8	*dileptus*	1.0	1.3	1.8	0.0
rozella	1.2	1.7	2.8	0.4	*parachlorella*	0.9	1.2	2.3	0.6
salpingoeca	1.2	1.6	0.2	2.4	*trechispora*	0.8	1.1	1.7	0.1
apodileptus	1.1	1.5	2.1	0.0	*phlyctochytrium*	0.8	1.0	1.5	0.0
oscheius	1.1	1.4	0.4	2.2	*rhogostoma*	0.7	1.0	1.1	1.8
hyaloraphidium	1.0	1.3	0.0	2.0	*steinia*	0.6	0.9	1.2	0.0
neochloris	1.0	1.3	2.2	0.4	*vorticellides*	0.6	0.9	0.4	1.2
sterigmatomyces	1.0	1.3	0.8	2.4	*stenostomum*	0.5	0.7	0.1	1.0

Table A15: Average relative abundance of toxin-producing, spoilage-causing, and plant-pathogenic bacterial, fungal and oomycete species in the hydroponic reservoirs.

Organism	Toxin production	Food spoilage	Plant pathogen	Algal grow beds			Bacterial grow beds		
				RA (%) Phase I	RA (%) Phase II	RA (%) Phase III	RA (%) Phase I	RA (%) Phase II	RA (%) Phase III
Bacterial species									
clostridium perfringens	+	+	-	0.01	0.00	0.01	0.00	0.00	0.03
clostridium botulinum	+	+	-	0.00	0.00	0.00	0.01	0.00	0.00
pseudomonas aeruginosa	+	+	-	0.00	0.04	0.04	0.00	0.00	0.00
vibrio cholerae	+	-	-	0.03	0.00	0.02	0.00	0.00	0.00
phormidium tenue	+	-	-	0.00	0.03	0.00	0.00	0.02	0.00
oscillatoria amphigranulata	+	-	-	0.00	0.00	0.00	0.00	0.26	0.00
phormidium autumnale	+	-	-	0.00	0.10	0.00	0.00	0.00	0.00
pseudanabaena mucicola	+	-	-	0.00	0.00	0.00	0.00	0.00	0.02
phormidium tergestinum	+	-	-	0.00	0.49	0.06	0.00	0.00	0.00
salmonella enterica	+	-	-	0.01	0.00	0.01	0.00	0.00	0.00
Fungal species									
penicillium namyslowskii	-	+	-	0.18	0.00	0.00	0.18	0.00	0.36
penicillium janthinellum	+	+	-	0.00	0.00	0.00	0.43	0.00	0.00
penicillium coccotrypicola	-	+	-	0.00	0.00	0.00	0.47	0.00	0.00
aspergillus flavus	+	+	+	0.00	0.00	0.00	1.64	0.00	0.00
rhizopus oryzae	-	+	-	23.74	0.35	0.35	7.28	1.49	0.29
ulocladium alternariae	-	+	+	1.73	0.00	0.00	0.00	0.00	0.00
Oomycetes species									
phytopythium boreale	-	-	+	0.00	0.00	0.00	0.00	0.04	0.00
pythium aphanidermatum	-	-	+	0.00	0.00	0.06	0.00	0.01	0.46

RA: Relative abundance

Table A16: Primers of target genes and thermocycling conditions used in qPCR

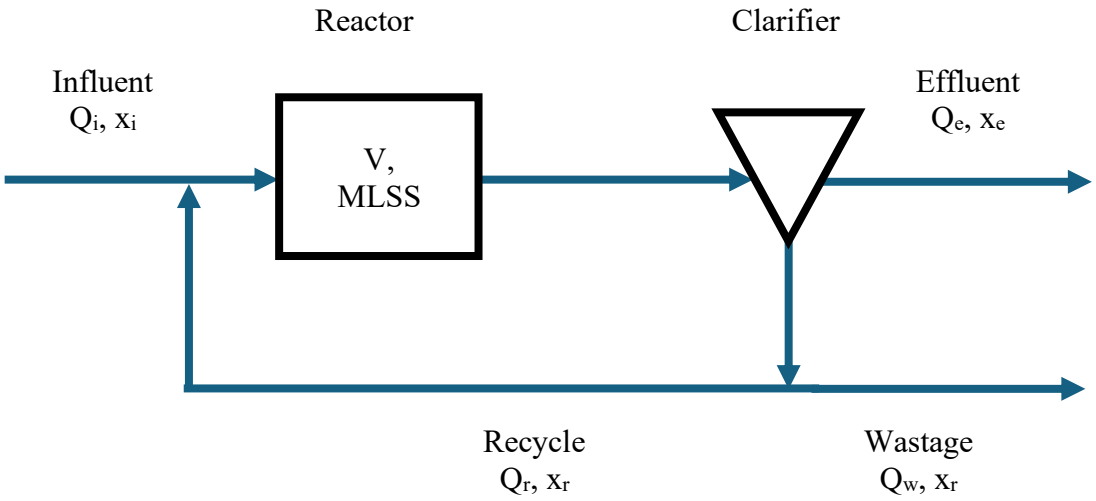
Target gene	Primer	Primer sequence (5' – 3')	Product length (bp)	Thermal profile	Reference
amoA	amoA-1F	GGGGTTTCTACTGGTGGT	490	Pre-denaturation at 95 °C for 5 mins, followed by 35 cycles of denaturation at 95 °C for 15 secs, annealing at 56 °C for 30 secs, and extension at 72 °C for 45 secs	[46,246]
	amoA-2R	CCCCTCKGSAAAGCCTTCTTC			
nxrB	nxrB169f	TACATGTGGTGGAACA	485	Pre-denaturation at 95 °C for 5 mins, followed by 35 cycles of denaturation at 95 °C for 15 secs, annealing at 53 °C for 30 secs, and extension at 72 °C for 45 secs	[46,247]
	nxrB638r	CGGTTCTGGTCRATCA			
nirK	nirK876_F	ATYGGCGGVCAYGGCGA	165	Pre-denaturation at 95 °C for 30 secs, followed by 40 cycles of denaturation at 95 °C for 5 secs, annealing at 55 °C for 40 secs, and extension at 72 °C for 50 secs	[248,249]
	nirK1040_R	GCCTCGATCAGRTTRTGGTT			
nirS	nirScd3aF	GTSAACGTSAAGGARACSGG	425	Pre-denaturation at 95 °C for 30 secs, followed by 40 cycles of denaturation at 95 °C for 5 secs, annealing at 57 °C for 30 secs, and extension at 72 °C for 40 secs	[249,250]
	nirSR3cd	GASTTCGGRTGSCTCTTGA			
nosZ	nosZf	AACGACAAGDYCAA	1433	Pre-denaturation at 95 °C for 3 mins, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 2 mins	[249,251]
	nosZr	AKSGCRTGGCAGAA			

Table A17: Top 20 prokaryotes at genus-level in bioreactors

genus	72-hr HRT						48-hr HRT					
	Algal R1	Algal R2	Algal R3	Bacterial R1	Bacterial R2	Bacterial R3	Algal R1	Algal R2	Algal R3	Bacterial R1	Bacterial R2	Bacterial R3
neolewinella	2.0±3.4	0.1±0.2	0.1±0.1	9.4±8.0	14.0±18.2	11.4±9.6	0.6±0.8	0.7±1.1	0.3±0.3	27.7±5.7	36.6±0.8	30.2±6.3
sphaerotilus	15.0±25.1	29.6±49.7	5.2±8.1	18.7±12.6	32.7±31.1	10.0±17.1	0.2±0.1	0.1±0.1	0.2±0.1	4.1±6.4	1.9±1.7	1.1±1.6
limnothrix	16.1±27.8	18.2±31.2	7.7±8.1	0.0±0.0	0.0±0.0	0.0±0.0	2.3±2.9	14.2±22.3	3.2±4.2	0.0±0.0	0.0±0.0	0.0±0.0
pseudacidovorax	3.6±3.8	7.1±11.2	5.7±8.6	2.1±0.3	1.3±0.6	0.8±0.3	2.7±0.6	1.1±1.5	0.8±0.6	5.4±5.0	7.8±9.0	7.9±11.0
aquabacterium	0.7±0.4	0.4±0.1	1.2±0.5	4.4±2.1	3.3±3.3	1.7±1.4	6.6±1.3	6.7±4.0	2.1±0.7	6.0±4.3	5.7±2.6	4.9±2.9
zoogloea	1.4±2.3	0.1±0.1	0.1±0.1	7.2±11.0	0.6±0.6	9.7±16.5	10.0±16.6	2.8±4.9	0.5±0.7	3.1±2.6	0.7±0.8	2.0±2.6
sulfuritalea	1.8±1.8	0.8±0.7	1.8±1.7	1.5±1.1	1.3±1.5	1.8±1.7	5.1±4.3	3.7±2.8	6.9±8.0	2.4±1.3	2.1±1.5	2.4±1.4
comamonas	5.6±7.1	2.8±3.1	3.3±2.1	2.1±0.9	1.8±0.6	0.8±0.3	1.9±0.7	1.5±1.0	1.1±0.2	2.0±1.3	1.7±0.6	1.5±0.1
acidovorax	5.3±4.4	3.8±4.2	3.8±3.6	2.3±1.1	1.3±1.0	0.8±0.6	2.1±0.9	1.0±0.5	2.0±0.4	1.8±1.7	1.8±1.4	2.4±2.8
brachymonas	7.1±12.1	5.3±9.0	5.7±9.5	1.8±2.6	0.4±0.3	0.3±0.1	0.3±0.1	0.4±0.2	0.3±0.1	5.0±8.0	0.5±0.2	0.6±0.1
ignavibacterium	2.4±2.4	0.3±0.2	0.3±0.1	5.3±5.0	1.7±2.5	2.0±2.7	3.1±3.1	5.2±4.2	2.4±0.2	0.5±0.3	0.3±0.4	0.3±0.3
sediminibacterium	1.1±1.8	0.3±0.5	0.1±0.1	4.5±4.4	4.0±3.0	2.6±2.8	0.7±0.2	1.1±1.1	0.5±0.4	3.9±0.6	2.5±1.5	3.0±2.0
hydrogenophaga	3.7±5.3	0.7±0.7	0.8±0.6	1.2±0.6	0.8±0.7	0.7±0.7	3.8±1.0	3.5±4.5	1.7±2.0	2.0±0.4	0.8±0.2	0.7±0.4
rhodocyclus	0.9±1.2	0.3±0.3	0.2±0.2	0.3±0.1	0.2±0.2	0.3±0.1	4.7±3.9	2.1±1.6	6.1±10.0	0.7±0.5	1.3±2.0	0.8±1.0
tabrizicola	1.5±1.0	0.7±0.6	0.6±0.4	0.3±0.2	0.1±0.1	0.1±0.1	5.7±2.1	3.4±3.4	1.2±0.9	0.8±0.7	0.6±0.1	0.4±0.2
limnohabitans	1.2±0.3	1.1±1.4	0.9±0.7	0.7±0.2	0.4±0.1	0.5±0.4	1.0±0.4	0.5±0.2	1.9±1.9	0.9±0.4	1.1±0.5	2.0±1.1
azohydromonas	0.3±0.4	0.2±0.1	0.6±0.3	1.6±0.7	1.3±0.1	0.8±0.7	1.5±0.7	1.6±0.9	0.6±0.1	1.2±0.7	1.7±0.7	1.5±0.9
nitrospira	0.1±0.1	0.3±0.2	2.4±3.1	0.9±0.1	0.5±0.5	1.5±0.6	0.5±0.7	0.3±0.3	1.7±0.7	0.2±0.1	1.7±1.7	2.4±2.1
rhabdaerophilum	0.1±0.1	0.1±0.1	0.1±0.1	1.5±2.4	0.2±0.3	4.0±6.9	2.7±4.3	1.1±1.8	0.2±0.2	0.2±0.2	0.4±0.6	0.4±0.5
georgfuchsia	0.2±0.1	0.2±0.2	0.9±0.2	0.4±0.2	0.3±0.3	0.5±0.2	1.6±1.0	1.0±0.9	1.8±0.8	1.4±0.7	0.7±0.2	0.9±0.2

Values are means ± standard deviation of 3 biological replicates.

Calculation of solids retention time in bioreactors



$$\text{SRT} = \frac{\text{Solids in reactor}}{\text{Solids wasted}}$$

$$\text{SRT} = \frac{\text{Solids in biofilm} + \text{Solids added through recycling}}{\text{Solids withdrawn (wasted)} + \text{Solids in effluent}}$$

$$\text{SRT} = \frac{(\text{V x MLSS}) + (\text{Q}_\text{r} \text{ x X}_\text{r})}{(\text{Q}_\text{w} \text{ x X}_\text{r}) + (\text{Q}_\text{e} \text{ x X}_\text{e})}$$

Assumption: No sludge wastage; $(\text{Q}_\text{w} \text{ x X}_\text{r}) = 0$

$$\text{SRT} = \frac{(\text{V x MLSS}) + (\text{Q}_\text{r} \text{ x X}_\text{r})}{(\text{Q}_\text{e} \text{ x X}_\text{e})}$$

Data

V	15 gal (56.78 L)
Q_r	5 gal (18.93 L)
Q_e	~65.5 L/day
MLSS (g/L)	8.68 (Algae); 2.42 (Bacteria)
X_r (g/L)	4.44 (Algae); 9.57 (Bacteria)
X_e (g/L)	0.17 (Algae); 0.11 (Bacteria)

SRT (Algal system) = 51.81 days
SRT (Bacterial system) = 44.21 days