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ชื่อนิสิต Miss Piyatida Hussanodart

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SENIOR PROJECT

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Student name Miss Piyatida Hussanodart **Student ID** 6033330223

Project adviser Dr. Sumeth Wongkiew

Department Environmental Science

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Evaluation of nitrogen concentrations in bioponics under acetic acid additions

By

Piyatida Hussanodart

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Name	Miss Piyatida Hussanodart	Student ID 6033330223
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Accepted by Department of Environmental Science, Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Degree of Bachelor of Science.

.... Head of Department of Environmental Science

(Assistant Professor Pasicha Chaikaew, Ph.D.)

PROJECT COMMITTEE

.... Chairman

(Associate Professor Nuta Supakata, Ph.D.)

.... Committee

(Assistant Professor Pasicha Chaikaew, Ph.D.)

.... Project Advisor

(Sumeth Wongkiew, Ph.D.)

หัวข้อ **การประเมินความเข้มข้นของไนโตรเจนในไบโอโพนิกส์ภายใต้การเติมกรดอะซิติก**
โดย นางสาว ปิยะธิดา หัสโนดาต **รหัสประจำตัวนิสิต** 6033330223
อาจารย์ที่ปรึกษา ดร. สุเมธ วงศ์เขียว
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บทคัดย่อ

ระบบไบโอโพนิกส์เป็นการทำงานร่วมกันระหว่างการปลูกผักแบบไฮโดรโพนิกและของเสียอินทรีย์ ซึ่งในระบบสามารถหมุนเวียนธาตุไนโตรเจนจากการใช้ปุ๋ยชีวภาพ ในระบบไบโอโพนิกส์มีจุลินทรีย์ที่สามารถเปลี่ยนสารอินทรีย์ให้อยู่ในรูปที่พืชสามารถใช้งานได้ การเปลี่ยนรูปไนโตรเจนในไบโอโพนิกส์มีความสำคัญอย่างยิ่งต่อประสิทธิภาพของพืชในการนำไนโตรเจนไปใช้ในการเจริญเติบโตในระบบไบโอโพนิกส์และลดการปล่อยของเสียสู่สิ่งแวดล้อม ในการศึกษานี้ได้มีการศึกษาชนิดของแบคทีเรียที่เชื่อมโยงกับการเปลี่ยนรูปของไนโตรเจนโดยการวิเคราะห์กลุ่มชุมชนจุลินทรีย์ ด้วยวิธี Next-Generation Sequencing ระบบไบโอโพนิกส์นี้ได้นำผักกรีนคอสมาเพาะปลูก (14 ต้นต่อระบบ) และใช้ปุ๋ยมูลไก่ 500 กรัมต่อระบบ เพื่อเป็นแหล่งอาหารของพืช การทดลองได้ทำการเติมกรดอะซิติก (5% w/v) สำหรับระยะที่ 1 (กรดอะซิติก 0 มล. เทียบกับกรดอะซิติก 700 มล.) และระยะที่ 2 (กรดอะซิติก 350 มล. เทียบกับกรดอะซิติก 1,050 มล.) ระยะที่ 3 (กรดอะซิติก 0 มล. 350 มล. 700 มล. และ 1,050 มล.) จากการศึกษาพบว่าการเติมกรดอะซิติกในระบบไบโอโพนิกส์ไม่มีมีผลต่อประสิทธิภาพของพืชในการนำไนโตรเจนไปใช้ แต่ในแง่ของการเจริญเติบโตของพืช พบว่าระบบที่ไม่มีการเติมกรดอะซิติกทำให้พืชเจริญเติบโตดีที่สุด นอกจากนี้ผลการวิจัยพบว่าระบบไบโอโพนิกส์มีค่าคุณภาพน้ำที่ดีต่อพืชและจุลินทรีย์ในการเจริญเติบโต โดยค่าคุณภาพน้ำมีดังนี้ ค่าออกซิเจนละลายน้ำ, พีเอช, อุณหภุมิ, แอมโมเนียรวม, ไนไตรท์, ไนเตรท และน้ำหนักของพืชเมื่อมีการเติมกรดอะซิติก ค่าที่ได้เป็นดังนี้ 6.6 ± 0.3 mg/L, 7.7 ± 0.1, 29.4 ± 1.8 °C, 3.0±0.6 mgN/L, 2.4 ± 0.7 mgN/L, 2.6 ± 1.0 mg/L, 921.5 ± 358.5 กรัม (กรดอะซิติกเข้มข้น 0 มล.); 6.7 ± 0.2 mg/L, 6.3 ± 0.7, 28.8 ± 1.3 °C, 2.8 ± 1.0 mgN/L, 2.3 ± 1.3 mgN/L, 3.0 ± 1.4 mg/L, 545.0 ± 339.4 กรัม (กรดอะซิติกเข้มข้น 350 มล.); 6.1 ± 0.4 mg/L, 6.1 ± 1.0, 29.5 ± 1.9 °C, 3.1 ± 1.3 mgN/L, 2.5 ± 1.6 mgN/L, 3.0 ± 1.5 mg/L, 402.5 ± 208.6 กรัม (กรดอะซิติกเข้มข้น 700 มล.); 6.9 ± 0.2 mg/L, 5.0 ± 0.3, 28.7 ± 1.4 °C, 2.6 ± 1.9 mgN/L, 3.9 ± 1.9 mgN/L, 3.0 ± 2.2 mg/L, 338.0 ± 299.8 กรัม (กรดอะซิติกเข้มข้น 1050 มล.) ตามลำดับ ซึ่งพบว่าการเติมกรดอะซิติกส่งผลที่ลดประสิทธิภาพการใช้ไนโตรเจน และการเติมกรดไปรบกวนการเจริญเติบโตของพืช นอกจากนี้ยังศึกษาพบกลุ่มของแบคทีเรียที่มีผลต่อวัฏจักรไนโตรเจนในระบบไบโอโพนิกส์ ดังนี้ แบคทีเรียกลุ่ม *Xanthobacteraceae* มีหน้าที่ในการเปลี่ยนก๊าซไนโตรเจนให้กลายเป็นแอมโมเนียรวมโดยผ่านกระบวนการตรึงไนโตรเจน แบคทีเรียกลุ่ม *Lentimicrobium* และ *Rhodobacteraceae* เปลี่ยนแอมโมเนียรวมให้เป็นไน

ไตรท์ด้วยกระบวนการแอมโมเนียออกซิเดชั่น แบคทีเรียกลุ่ม *Nitrosomonadaceae* และ *Proteobacteria* เปลี่ยนไนไตรท์ให้เป็นไนเตรท ด้วยกระบวนการไนไตรท์ออกซิเดชั่น และแบคทีเรียกลุ่ม *Denitratisoma* และ *Microscillaceae* ทำการเปลี่ยนไนเตรทให้กลับสู่รูปก๊าซไนโตรเจนผ่านกระบวนการดีไนตริฟิเคชั่น ดังนั้น การศึกษาทำให้ทราบว่าไบโอโพนิกส์มีประสิทธิภาพในการหมุนเวียนธาตุอาหารจากปุ๋ยอินทรีย์โดยมีกรดอะซิติกไปลดการเจริญเจิบโตของพืช

คำสำคัญ: กรดอะซิติก, กลุ่มจุลินทรีย์, ไบโอโปนิกส์, ปุ๋ยมูลไก่, ไนโตรเจน

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Project advisor	Dr. Sumeth Wongkiew		
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Academic year	2020		

Abstract

The bioponic system is a combination between hydroponic vegetable cultivation and organic waste. The system can circulate nitrogen elements from the use of bio-fertilizers. In bioponic systems, there are microorganisms that can convert organic matter into a usable form of plants. Nitrogen transformation in bioponics is critical to the plant efficiency in utilizing nitrogen for growth in bioponic systems and reducing emissions to the environment. In this study, bacterial species linked to nitrogen transformation was studied by analyzing the microbial community groups using Next-Generation Sequencing. 14 plants per system) and 500 grams of chicken manure were used as a food source for plants. The system was added to acetic acid (5% w/v) for Phase I (0 ml acetic vs 700 ml acetic acid) and Phase 2 (350 ml acetic vs. Acetic acid 1050 ml.) Phase 3 (acetic acid 0 ml. 350 ml. 700 ml. and 1,050 ml.) From this study, it was found that adding acetic acid to the bioponic system had negative effect on the plant efficiency of nitrogen utilization and also plant growth. It was found that the system without the addition of acetic acid gave the best plant growth. In addition, the results of the research showed that the bioponic system had good water quality for plants and microorganisms for growth. The water quality parameters were dissolved oxygen, pH, temperature, total ammonia, nitrite, nitrate and plant weight. The values were as follows: 6.6 ± 0.3 mg /L, 7.7 ± 0.1, 29.4 ± 1.8 °C, 3.0 ± 0.6 mgN/L, 2.4 ± 0.7 mgN/L, 2.6 ± 1.0 mgN/L, 921.5 ± 358.5 g (acetic acid concentration 0 ml); 6.7 ± 0.2 mg/L, 6.3 ± 0.7, 28.8 ± 1.3 °C, 2.8 ± 1.0

mgN/L, 2.3 ± 1.3 mgP/L, 3.0 ± 1.4 mgN/L, 545.0 ± 339.4 g (acetic acid concentrate 350 ml); 6.1 ± 0.4 mg/L, 6.1 ± 1.0, 29.5 ± 1.9 °C, 3.1 ± 1.3 mgN/L, 2.5 ± 1.6 mgN/L, 3.0 ± 1.5 mgN/L, 402.5 ± 208.6 g (acetic acid concentration 700 ml); 6.9 ± 0.2 mg/L, 5.0 ± 0.3, 28.7 ± 1.4 °C, 2.6 ± 1.9 mgN/L, 3.9 ± 1.9 mgN/L, 3.0 ± 2.2 mgN/L, 338.0 ± 299.8 g (acetic acid concentration 1050 ml) respectively, which showed that adding acetic acid had a negative effect on nitrogen utilization efficiency. Also, the addition of acid reduced plant growth. In addition, groups of bacteria such as *Xanthobacteraceae* have been found to influence the nitrogen cycle in the bioponic system. It is responsible for the conversion of nitrogen gas to total ammonia through nitrogen fixation process. Groups of bacteria *Lentimicrobium* and *Rhodobacteraceae* converted total ammonia to nitrite with ammonia oxidation process. This study found groups of bacteria *Nitrosomonadaceae* and *Proteobacteria* (converting nitrite into nitrate with nitrite oxidation process) and bacterial groups *Denitratisoma* and *Microscillaceae*, which are responsible for process that nitrate is converted back to nitrogen gas through denitrification process. Therefore, the study showed that bioponics was effective in the nutrient circulation from organic fertilizers; however, high concentration and loading rate of acetic acid could inhibited plant growth.

Keywords: Acetic acid, Bioponics, Chicken manure, Nitrogen transformation, Microbial community

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CHAPTER 1

INTRODUCTION

1.1 Overviews

Wastewater containing residuals high concentrations of nitrogen can cause oxygen depletion and eutrophication in the receiving water bodies (Calone et al. 2019). Bioponics is a way to utilize organic residue especially manures (e.g., chicken manure) as a source of fertilizer for vegetables in a recirculating system. Bioponics is a controlled growing system in which plants in growing media take up nutrients from plant-based, animal-based and mineral natural substances released by the biological activity of microorganisms (Fang & Chung, 2017). Bioponics consists of two main parts namely grow bed and biofilter, supporting each other with microorganisms living in both plant roots in grow bed and manure zone in biofilter. In plant roots and biofilter, microorganisms change ammonia to nitrite and nitrate, respectively. Microorganisms help to maintain nitrogen and solubilize nutrients for vegetables grow. In the biofilter of bioponics, organic fertilizers (e.g., organic compost, chicken manure) are degraded by the microbes and then continuously release nutrients (e.g., nitrogen and phosphorus) for plant uptake in grow beds. A biofilter is necessary for bioponics. Biofilter also remove suspended solids and keeps plant microbe ecosystems.

A key advantage of bioponics is the symbiotic relationship between the plants and the microorganisms of the horticultural and biofilter systems, respectively, which can be connected through the recirculation of the water flow. The degradation of organic manure can provide nutrients for the plant growth in the horticultural one, whereas plants, in turn, clean and filter the water that can be reused back to leach nutrient in biofilter and release nutrient over and over again. This symbiotics depends on the action of two different groups of bacteria, namely ammonia and nitrite oxidizing bacteria (Wongkiew, Hu, et al. 2017). These bacteria oxidize the ammonia and nitrites leached from layer of organic manure in biofilter to nitrates, which are easier to absorb by the plant roots (Rakocy et al., 2006). When an appropriate balance between the nitrogen generation by biofilter and the plants' nutrient uptake is achieved, nitrogen can be recovered from waste to vegetable highly efficiently.

Accordingly, the discharge of manure waste into the environment can be reduced by the application of bioponic systems.

Recently, there are studies about using compost mixed with animal manure applied with microorganisms in hydroponics and determining the impact of manure-based extracts on plant yield in hydroponics (Drozdz et al., 2020). Bio-fertilizer contains essential nutrients for plants, such as nitrogen and phosphorus. Bio-fertilizers made from chicken manure can be added as a nutrient source to bioponics systemic due to its high nutrient content.

The chemical composition of bioponic nitrogen is complex because of a large number of dissolved ions and organic substances resulting from the release of excretory compounds from waste in biofilter. The interaction between the main ions in solution can influence the chemical composition of bioponic nutrient solutions. Nitrogen is an essential element for plants in bioponics, as it is used as an indicator to evaluate the nutrient efficiency of bioponic systems. Nitrogen is a macronutrient that plants use in many production enzyme, cell structure and plant biomass. In bioponics, organic nitrogen in the organic compost is degraded to form ammonia nitrogen (NH_4^+). The ammonia nitrogen is converted to nitrite (NO_2^-) to nitrate (NO_3^-) via nitrification process. Then, the nitrate is recycled as a fertilizer for plant growth in the hydroponic grow bed (Wongkiew, Hu, et al. 2017). The main process that transforms NH_4^+ to NO_3^- in the presence of oxygen is nitrification. Particularly in biofilters, a main a main pathway contributing to nitrogen loss due to anoxic condition in biofilter is denitrification, which converts both NO_3^- to NO_2^-, nitric oxide (NO) and finally to nitrogen gas (N_2) by denitrifying bacteria (denitrifiers) under anoxic condition (low dissolved oxygen conditions). As it can be seen, microorganisms are the important key that drives nitrification and denitrification in bioponic systems. Thus, concentrations of different forms of nitrogen and nitrogen transformations in bioponics could be affected by the microbial community, which could contribute to bioponic performance such as plant productivity.

pH was found to have effects on the uptake of nutrients by plant roots (Cerozi and Fitzsimmons 2016). Maintaining or regulating pH is critical to control nitrogen availability for plants. This is because the pH of the solution is a value that indicates the root's ability to absorb the nutrients contained in the plant nutrient solution. pH could increase the nitrogen solubilizing rate of manure in biofilters due to the increase in enzymatic reactions under an optimal pH. For example, microbial nitrification of ammonia to nitrite and nitrite to nitrate is optimized at pH 8.5, but plant nutrient uptake for many crop species is optimized near pH 6.0. Thus, pH in bioponics systems can be maintained at pH 7.0 to support the plant growth (Cerozi and Fitzsimmons 2016). Normally, the pH of 5.8-7.0 should be maintained because it is a suitable range for the nutrients of various plants that can remain in solution. That plants can utilize effectively. However, there is no studies that demonstrate the positive or negative impacts of adding acids for maintain a certain pH level in bioponic systems. Adjustments to reduce pH can be made by adding additives to plant nutrient solutions such as sulfuric acid (H_2SO_4), nitric acid (HNO_3), hydrochloric acid (HCl) or acetic acid. Among them, acetic acid is the most possible to claim bioponics as organic production because acetic acid is weak acid which is organic acid, while the others are inorganic acid that are not used in organic productions (Cerozi and Fitzsimmons 2016).

Research on plant growth under acetic acid additions for bio-stimulation/inhibition of nitrogen concentration in bioponic systems is not widely evaluated. And no studies have been done on the addition of acids to enhance the efficiency of bioponic systems. Thus, the overarching goal of this study is to develop a compost-based bioponic system and evaluate the effects of adding acetic acid on nitrogen concentrations and microbial community under different acetic acid loading rates. Comparison of microbial communities under acetic acid addition and without acetic acid addition will be conducted. The finding could be helpful in recommendation an efficient bioponic systems by adding or not adding acetic acids.

1.2 Research Objectives

1.2.1 To evaluate nitrogen concentrations in bioponic systems under different acetic acid loading rates

1.2.2 To compare microbial community between bioponics with acetic acid addition and without acetic acid addition

1.3 Expected Outcomes

1.) Nitrogen concentrations and plant growth in bioponics system at different acetic acid loading rates.

2.) Recommendations to grow organic vegetables in bioponics by adding acetic acid.

3.) Key microbes found in the bioponics system under the acetic acid addition and without acetic acid addition.

CHAPTER 2

LITERATURE REVIEWS

2.1.1 Effects of pH in nutrient availability

The pH in a solution is important for nitrogen uptake by plants because of the root's ability to absorb nutrients depends in pH levels. In the plant nutrient solution, the pH value of 5.8-7.0 should be maintained for efficient uptake by plants. The pH of the nutrient solution can change after plants exchanges ions. For example, pH can change when there is an absorption of nutrients in the nutrient solution at which. Plants release hydrogen (H^+) and hydroxide (OH^-) from the roots into a nutrient solution, causing the pH to change. Absorption of negative ions or anions such as nitrates ($NO3^-$), sulfates (SO_4^{2-}), phosphates (PO_4^{3-}) will release hydroxide (OH^-) into nutrient solution. Positive electric charges or cations (cations) such as calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^+), ammonium (NH_4^+) will release hydrogen (H^+) into nutrient solutions. Plant nutrient solution already has a positive electric charge or cation than the value of anion. Thus, the pH will decrease after plants absorb positively-charged nutrient solution. For some nutrients that plants need to use in large quantities, nitrogen (nitrogen, N), which is supplied in the forms of nitrates (NO_3^-) and ammonium (NH_4^+), the ratio of these substance must be carefully considered because it has a great influence on the change in pH and utilization of plants.

2.1.2 Composting and its characteristics

Aerobic composting is an effective approach for the treatment of livestock Manure. Animal manure is rich in organic matter, and manure can be fully utilized by microorganisms, resulting in stable products that are beneficial to the growth of plants. However, about 10% of the nitrogen can be emitted into the atmosphere as N_2O during the process of aerobic composting (Wu et al., 2012). Animal manure is a valuable resource, which contains a lot of nutrients including nitrogen, phosphorus, and organic matter (Drozdz et al., 2020). Aerobic composting is an effective way to reduce, render harmless and recycle husbandry manure, which could sufficiently convert manure into organic fertilizer, and has been causing international attention (Kong et al.,2018; Awasthi et al., 2020).

Chicken manure is the feces of chickens used as an organic fertilizer. Of all animal manures, and it has the highest amount of nitrogen, phosphorus, and potassium. Chicken manure consists of both organic and inorganic forms of the plant nutrients. Nitrogen nutrient occurs as ammonia and uric acid. The uric acid converts to urea, the urea rapidly decomposes to ammonia gas, which causes the strong offensive odor. Phosphorus is primarily organic and becomes available as the manure decompose. Chicken manure are rich in nutrients, such as 18.7% crude protein, 2.5% adipose, 13% ash content, 11% carbohydrate and 7% fiber, including 2.34%, nitrogen, 2.32% phosphorus and 0.83% potassium (Molaey et al. 2018).

Figure 2.1 Chicken manure (https://www.agrifarming.in/chicken-manure-composting-process-benefits).

2.1.3 Biofilters and grow beds

Biofilters are one of the popular methods for treatment of nitrogen waste in wastewater systems. Several microbes perform microbial activities such as organic degradation process, nitrification process, and denitrification process. Nitrification is functioned by nitrifying bacteria under aerobic condition, and denitrification is functioned by denitrifying bacteria under anoxic condition. Organic degradation is functioned by heterotrophic bacteria both in aerobic and anaerobic condition. These bacteria grow and attached on biological filter material (Nelson, 2008).

In order to stimulate bacteria to grow in sufficient quantities in biofilter to fully react, organic additions (such as adding acetic acid) need to be added as a source of carbon for the bacteria. In the event that the concentration of the carbon source in the wastewater is insufficient for the denitrification process. There must also be a control of pH or acid/base addition over the operating time. To achieve a complete satisfied process and output, the control and regulation of acid-base addition in a certain range could be applied.

There several applications of plants for the treatment of organic matter and nutrients, especially nitrogen and phosphorus, in wastewater. Growing plants for nutrient removal in wastewater or waste stream is the method that has been widely popular because it is a treatment process that uses less energy and is environmentally friendly. Grow bed supplied with nutrients (bio-augmentation) including nitrogen compounds such as ammonia or nitrate is the way to treat the wastewater with simultaneous production of plants especially vegetables. This allows the primary nutrient for plants to absorb these substances for use in growth. In addition, nitrifying bacteria that live in hydroponic growing troughs and coexist with plant roots function as a biological filtration system. By converting ammonia into nitrate keeping the water clean enough to be reused and recirculated in the systems. The wastewater is be treated. Farmers can also find vegetables that are grown as an additional income.

The Nutrient Film Technique (NFT) system (Figure 2.2). It is another technique that has received much attention, with the principle that plant roots are immersed in tubular channels with pots supporting the roots that are slope-adjusted. Approximately 2 percent of the plant nutrient solution must be pumped from the plant nutrient solution storage tank, letting water flows into a thin sheet through the plant roots, the roots receive adequate oxygen. At the end of the trough, there will be a trough supporting the used plant nutrient solution to the tank for reuse (Saijai et al., 2016).

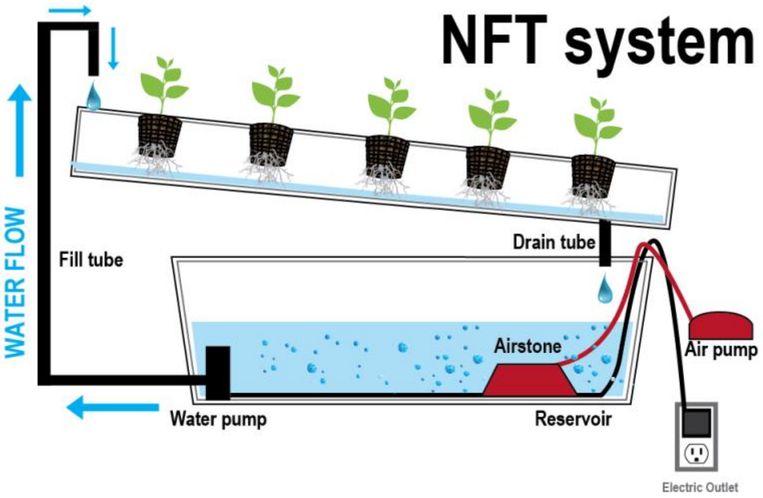


Figure 2.2 Nutrient Film Technique (NFT)

(Available from: http://greenbookpages.com)

2.1.4 Nitrogen assimilation by plant and nitrogen transformations by microbes

Plants absorb nitrogen from the soil and water in the form of nitrate (NO_3^-) and ammonium (NH_4^+). In aerobic environment where nitrification can occur, nitrate is usually the predominant form of available nitrogen that is absorbed by plants. However, this is not always the case as ammonia can predominate in flooded and anaerobic conditions. Plant roots themselves can affect the abundance of various forms of nitrogen by changing the pH and secreting organic compounds or oxygen. This influences microbial activities like the inter-conversion of various nitrogen species, the release of ammonia from organic matter in the sediments and organic matter and the nitrogen transformations of nitrifying and denitrifying bacteria. For plant growth, nitrate reduction is carried out in two steps in plant after absorption of nitrogen from soil and nutrient solutions. Nitrate is first reduced to nitrite (NO_2^-) in the cytosol by nitrate reductase using NADH or NADPH. Nitrite is then reduced to ammonia in the chloroplasts (in roots) by a ferredoxin dependent nitrite reductase. In photosynthesizing tissues, it uses an isoform of ferredoxin (Fd1) that is reduced by photosystem I while in the root it uses a form of ferredoxin (Fd3) that has a less negative midpoint potential and can be reduced easily by NADPH. In non-photosynthesizing tissues, NADPH is generated by glycolysis and the pentose phosphate pathway (Wongkiew, Hu, et al. 2017).

In bacteria, heterotrophs (organic carbon utilizers) assimilate ammonium and nitrate in the present of organic carbon for cell growth, and turn into cells, that reduce the nitrogen availability for plant uptake. Nitrifying microorganisms (autotrophs, inorganic carbon utilizers) in biofilters is essential to steadily oxidize ammonium and nitrite to nitrate. Nitrifying bacteria assimilate lower ammonium amount to their cells in comparison to heterotrophs. High concentration of heterotrophs in biofilters drastically lowers the dissolved oxygen (DO) and promotes anoxic condition, which causes denitrification and nitrogen loss via N_2. DO decreases in biofilters and around the root zone of plants due to the activities of aerobic microorganisms (e.g., nitrifiers and heterotrophs). Therefore, several nitrogen forms and DO affects nitrification efficiency, nitrogen emissions, and nitrogen availability for plant uptake (Wongkiew, Hu, et al. 2017).

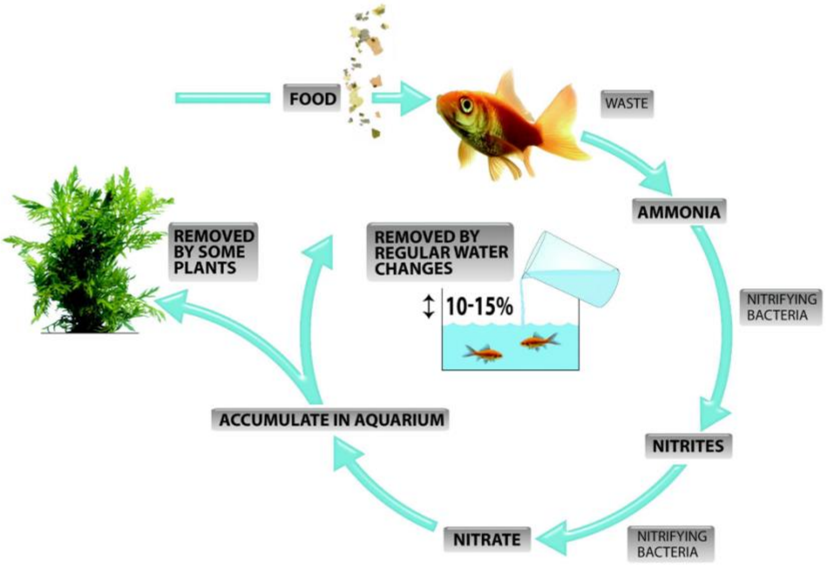


Figure 2.3 Biological nitrogen cycle (Available from: https://www.kingbritish.co.uk/blog/2015/10/what-is-the-nitrogen-cycle)

2.2 Relevant systems: Hydroponics and aquaponics

Hydroponics is the cultivation of plants without using soil. Hydroponic flowers, herbs, and vegetables are planted in inert growing media and supplied with nutrient-rich solutions, oxygen, and water. This system fosters rapid growth, stronger yields, and superior quality. When a plant is grown in soil, its roots are perpetually searching for the necessary nutrition to support the plant. If a plant’s root system is exposed directly to water and nutrition, the plant does not have to exert any energy in sustaining itself. The energy the roots would have expended acquiring food and water can be redirected into the plant’s maturation. As a result, leaf growth flourishes as does the blooming of fruits and flowers. Aquaponics is a sustainable method of raising both fish and vegetables. It is popular with individuals, entrepreneurs, educators, missions and governments. Furthermore, with this type of indoor farming, you grow substantially more food with less water, land and labor than traditional agriculture (Duarte et al. 2019).

CHAPTER 3

MATERIALS AND METHODS

3.1 Systems setup

The experiment operated at a terrace of the General Science building, Department of Environment Science, Faculty of Science, Chulalongkorn University. Nutrient film technique (NFT) bioponic systems were used as grow beds for this research (Figure 3.1). A bioponic system consisted of one recirculating tank for aeration and recirculation water (~18 liters), one upflow biofilter (~18 liters), and two-channel grow bed (14 plants per one bioponic system) (Figure 3.1). Dry chicken manure was applied in the biofilter and was used as a nutrient source for the systems because chicken manure had high concentrations of phosphorus, nitrogen and organic carbon, which are essential or plants and microbes (Ravindran et al., 2017). Cos Lettuce (*Lactuca sativa*), aka Cesar salad, was used as tested vegetables in this study. Cos Lettuce is one of the most popular fresh organic vegetables (Demir, 2019). Biochemical filter pad was use to increase surface area for microbial attachment in the up-flow biofilter. The bioponic systems were operated in duplicate (n=2).

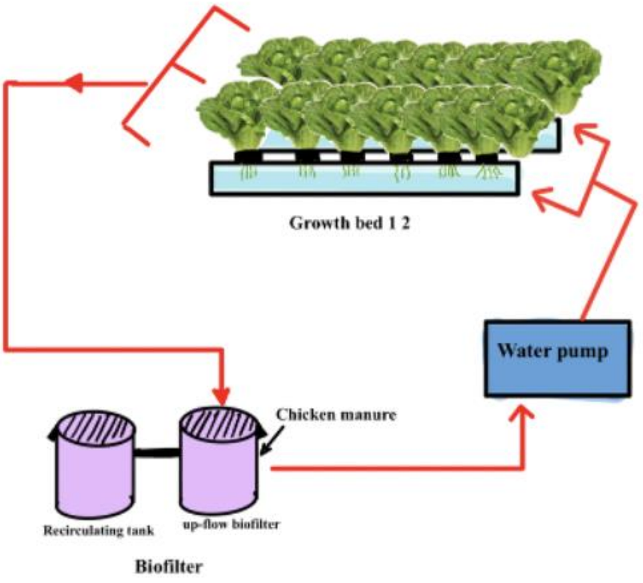
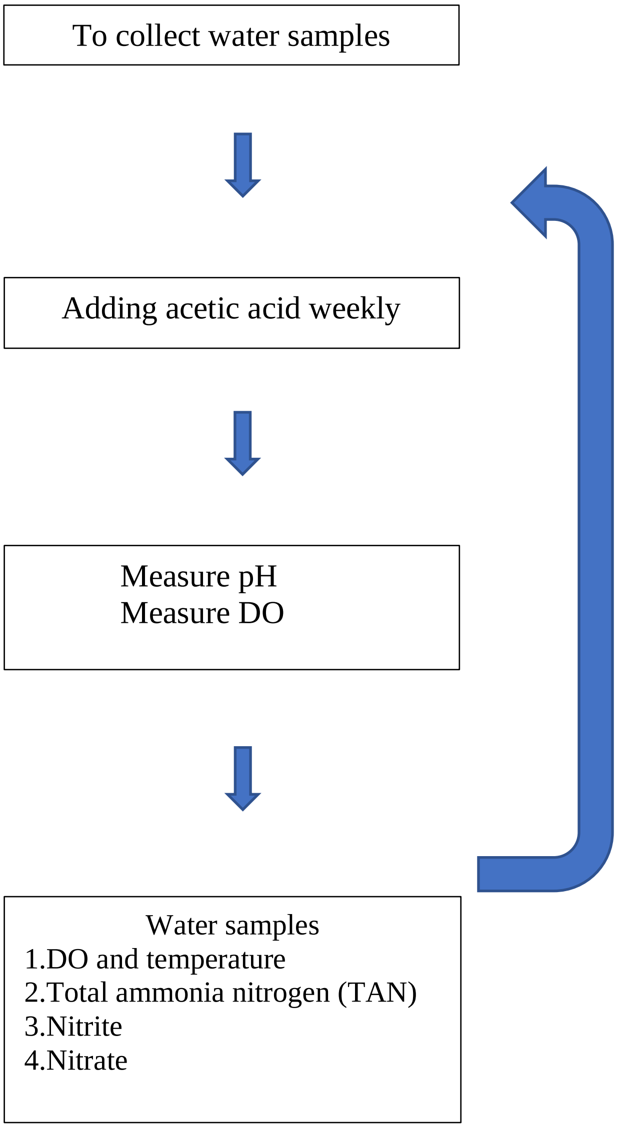


Figure 3.1 Diagram of a floating-raft bioponic system performed in this research.

3.2 Experimental design

For this study, there were 3 experimental runs of NFT bioponic systems (14 lettuces) by planting 3 times. After seed germination for about 1 weeks, plants were transferred to grow bed of bioponics (14 plants per system). Each plant cycle in bioponics required 5 weeks. At the beginning of each phase, 500 grams chicken manure compost per system were added with acetic acid (5% w/v) for phase 1 (0 mL acetic acid vs. 700 mL acetic acid, n =2) and phase 2 (350 mL acetic acid vs. 1050 mL acetic acid, n =2), phase 3 (acetic acid of 0, 350, 700 and 1050 mL, n =1). Water samples were collected every week. Each experiment was conducted in duplicate (n=2) with chicken manure 500 grams.



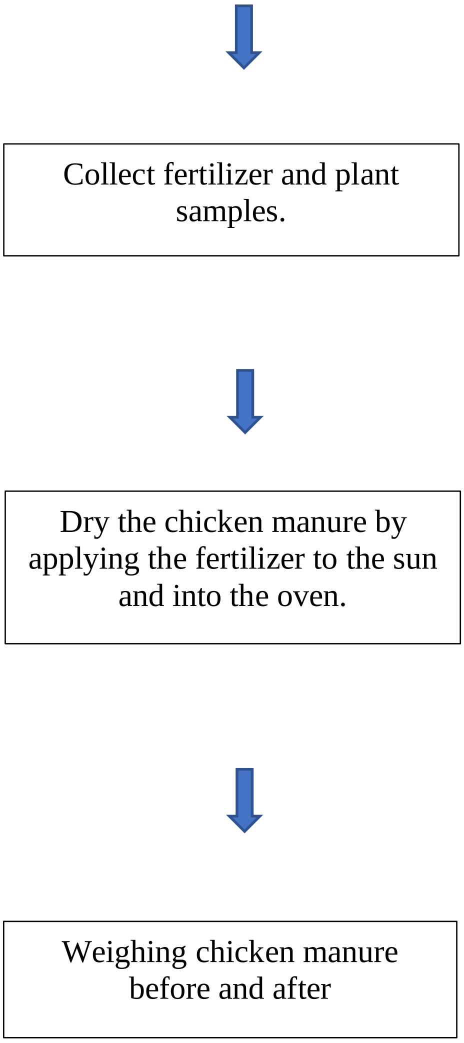


Figure 3.2 Diagram of experiments

Table 3.1 Experimental design

Weekly acetic acid	Experiment 1	Experiment 2	Experiment 3
Dose 1 (mL acetic acid)	0	350	0
Dose 2 (mL acetic acid)	700	1050	350
Dose 3 (mL acetic acid)	0	350	700
Dose 4 (mL acetic acid)	700	1050	1050

Calculation

1. The mass balance of N in bioponics

$$C_N.M_c = (C_{TAN}+C_{NO2\text{-}N}+C_{NO3\text{-}N})\,V + N_{plant} + N_{gas} + N_{precp} \quad (1)$$

Where, C_{TAN}, $C_{NO2\text{-}N}$, and $C_{NO3\text{-}N}$ are the concentrations of TAN, NO_2-N, and NO_3-N in recirculating water (g N/L), respectively; V is the volume of recirculating water (L); N_{plant} is the average N assimilated in plants at harvest (g N), respectively; T is the production duration (days); and N_{gas}/T is the rate of N loss (g N/day) via denitrifcation, respectively (Cerozi and Fitzsimmons, 2017; Wongkiew et al., 2017).

2. Nitrogen use effectively efficiency (NUE) in bioponics

$$NUE = \frac{Nplant}{Cn \cdot Mc \cdot T} \quad (2)$$

Where, C_N is the fractions of N in chicken manure solutions (g N/L), respectively; M_c is the chicken manure application rate (L/day); T is the production duration (days); N_{plant} and is the average N assimilated by plants at harvest (g N), respectively.

3.3 Laboratory Analysis

Water samples

1. **DO and temperature**

DO and temperature in bio-fertilizer tanks were monitored using the DO meter.

2. **Total ammonia nitrogen (TAN)**

Ammonia nitrogen was analyzed weekly using a nesslerization method, were calculated based on linear equation (y = mx + c) from a standard curve generated at the 410 nm. The equations can be obtained from a linear standard graph that is generated from series of standard dilution.

3. **Nitrite (NO_2^-)**

Nitrite nitrogen concentrations were analyzed weekly using a colorimetric method, calculated based on linear equation (y = mx + c) from a standard curve generated at the 543 nm. The equations can be obtained from a linear standard graph that is generated from series of standard dilution.

4. **Nitrate (NO_3^-)**

Nitrate nitrogen concentrations were analyzed weekly using a colorimetric method, calculated based on linear equation (y = mx + c) from a standard curve generated at the 410 nm. The equations can be obtained from a linear standard graph that was generated from series of standard dilution.

3.4 Microbial methods

Microbial community analyses were analyzed by next-generation sequencing (NGS, Illumina sequencing platform) targeting 16S rRNA genes (V3-V4 region), which were specific for each microbial genus. The relative abundances of microorganisms were calculated based on sequence reads from NGS data and used Greengenes database 13.8 for taxonomy classification. The microbial samples were analyzed using QIIME2 software by Omics Center, Faculty of Science, Chulalongkorn University. Microbial samples were taken from plant roots and sediment of chicken manure at the end of phase 1 to compare the microbial community between acid (loading rate = 700 mL/week) and control conditions.

3.5 Statistical Analysis

Statistical analyses were carried out using T-test (between two groups, α=0.05) with in each phase for making comparison of mean and for identifying significant difference ($p < 0.05$)

CHAPTER 4
RESULTS AND DISCUSSION

4.1. Bioponic system performance

The bioponic systems under acetic acid additions in closed recirculation hydroponic systems had a good performance. Bioponic systems from phases 1 to 3 worked at effective performance after adding the right amounts of acetic acid (350 – 700 mL) but decreased in growth rate. The plants grew effectively and had suitable weight (635 – 696 g/14 plants). The water in bioponic system had high dissolve oxygen (DO) concentration (6.1-6.9 mg/L) and warm temperature (28.7 – 30.9°C). Total ammonia nitrogen, nitrite, and nitrate concentrations were sufficient for the plant growth (Table 4.1). Overall, no significant difference of nitrogen concentrations was found when comparing two conditions within each phase by t-test, alpha = 0.05. However, the results in Table 4.1 show that NUE and plant growth decreased with the increase in acetic loading rates although significant difference was not found, suggesting that more replications (or more repeats of experiments) should be conducted to reduce the uncertainty or unexpected biological interferences. However, phase 3, which is a re-check phase, shows the confirmation that plant growth and NUE were negatively affected by the increase of acetic loading rates, which supported the results of phases 1 and 2.

In phases 1, the bioponic systems in this study showed bioponics with acetic acid addition (700 mL) and bioponic system without acetic acid addition (0 mL). From Table 4.1, bioponic system without acetic acid addition (0 mL) was found that TAN, nitrite and nitrate concentrations were 3.0 ± 0.6 mgN/L, 2.4 ± 0.7 mgN/L and 2.6 ± 1.0 mgN/L, respectively. And bioponic system with acetic acid addition (700 mL) was found that TAN, nitrite and nitrate concentrations were 3.1 ± 1.3 mgN/L, 2.5 ± 1.6 mgN/L and 3.0 ± 1.5 mgN/L, respectively. These results showed that nitrogen transformations under acetic acid additions better than the acid-free condition. Water quality parameters such as TAN, nitrite and nitrate concentrations in the bioponic systems were within the recommended range for effective nitrification and plant growths. The temperature in the bioponics ranged 29.4 – 29.5 ^{o}C.

In phases 2, the bioponic systems in this study showed bioponic system with acetic acid addition 350 mL and 1050 mL. From Table 4.1, bioponic system with acetic acid addition (350 mL) was found that TAN, nitrite and nitrate concentrations were 2.8 ± 1.0 mgN/L, 2.3 ± 1.3 mgN/L and 3.0 ± 1.4 mgN/L, respectively. And bioponic system with acetic acid addition (1050 mL) was found that TAN, nitrite and nitrate concentrations were 2.6 ± 1.9 mgN/L, 3.9 ± 1.9 mgN/L and 3.0 ± 2.2 mgN/L, respectively. These results showed that the addition of acetic acid at concentrations of 700mL and 1050 mL did not differ significantly in terms of water quality. But they differ clearly in terms of plant weight and NUE although they did not significantly different after performing t-test at level of confidence at 95% or significant level alpha of 0.05. Due to the excessive addition of acid, the value of too low (unsuitable) pH affected the growth of plants.

In phases 3 (checking phase), the bioponic systems in this study showed bioponic system with acetic acid addition 0 mL, 350, 700 and 1050 mL. From Table 4.1, bioponic system without acetic acid addition (0 mL) was found that TAN, nitrite and nitrate concentrations were 3.7 ± 0.8 mgN/L, 2.0 ± 0.5 mgN/L and 5.4 ± 2.7 mgN/L, respectively. Bioponic system without acetic acid addition (350 mL) was found that TAN, nitrite and nitrate concentrations were 3.6 ± 0.9 mgN/L, 6.7 ± 9.2 mgN/L and 5.0 ± 1.1 mgN/L, respectively, bioponic system without acetic acid addition (700 mL) was found that TAN, nitrite and nitrate concentrations were 3.9 ± 0.8 mgN/L, 2.7 ± 2.4 mgN/L and 4.9 ± 1.8 mgN/L, respectively. And bioponic system with acetic acid addition (1050 mL) was found that TAN, nitrite and nitrate concentrations were 2.9 ± 0.9 mgN/L, 3.2 ± 4.7 mgN/L and 6.9 ± 2.2 mgN/L, respectively. These results showed that the addition of acetic acid at concentrations of 0 mL, 350 mL, 700 mL and 1050 mL did not differ significantly in terms of water quality. But they differ clearly in terms of plant weight and NUE although they did not significantly different after performing t-test at level of confidence at 95% or significant level alpha of 0.05. Due to the excessive addition of acid, the value of too low (unsuitable) pH affected the growth of plants.

Table 4.1

Water Quality parameters (nitrogen and DO concentrations and TAN, NO_2^-, NO_3^- concentrations) in bioponic systems.

	Phase 1 (n= 12)		Phase 2 (n=12)		Phase 3 (check phase) (n=6)			
	0 mL	700 mL	350 mL	1050 mL	0 mL	350 mL	700 mL	1050 mL
TAN (mgP/L)	3.0 ± 0.6	3.1 ± 1.3	2.8 ± 1.0	2.6 ± 1.9	3.7 ± 0.8	3.6 ± 0.9	3.9 ± 0.8	2.9 ± 0.9
Nitrite (mgP/L)	2.4 ± 0.7	2.5 ± 1.6	2.3 ± 1.3	3.9 ± 1.9	2.0 ± 0.5	6.7 ± 9.2	2.7 ± 2.4	3.2 ± 4.7
Nitrate (mg/L)	2.6 ± 1.0	3.0 ± 1.5	3.0 ± 1.4	3.0 ± 2.2	5.4 ± 2.7	5.0 ± 1.1	4.9 ± 1.8	6.9 ± 2.2
pH	7.7 ± 0.1	6.1 ± 1.0	6.3 ± 0.7	5.0 ± 0.3	8.0 ± 0.3	6.8 ± 1.0	6.0 ± 1.6	5.2 ± 0.9
DO (mg/L)	6.6 ± 0.3	6.1 ± 0.4	6.7 ± 0.2	6.9 ± 0.2	6.5 ± 0.4	6.5 ± 0.2	5.8 ± 0.8	6.7 ± 0.3
Temp (deg C)	29.4 ± 1.8	29.5 ± 1.9	28.8 ± 1.3	28.7 ± 1.4	30.6 ± 1.8	30.9 ± 1.6	30.6 ± 1.7	30.7 ± 1.6
Plant wt. (g)	921.5 ± 358.5	402.5 ± 208.6	545.0 ± 339.4	338.0 ± 299.8	1194.0	696.0	635.0	522.0
NUE (%)	17.8 ± 7.0	7.0 ± 4.7	10.6 ± 6.5	6.6 ± 6.0	23.7	13.9	12.4	10.3

Note: No significant difference of nitrogen concentrations was found when comparing two conditions within each phase by t-test, alpha = 0.05.

4.2. Nitrogen transformations

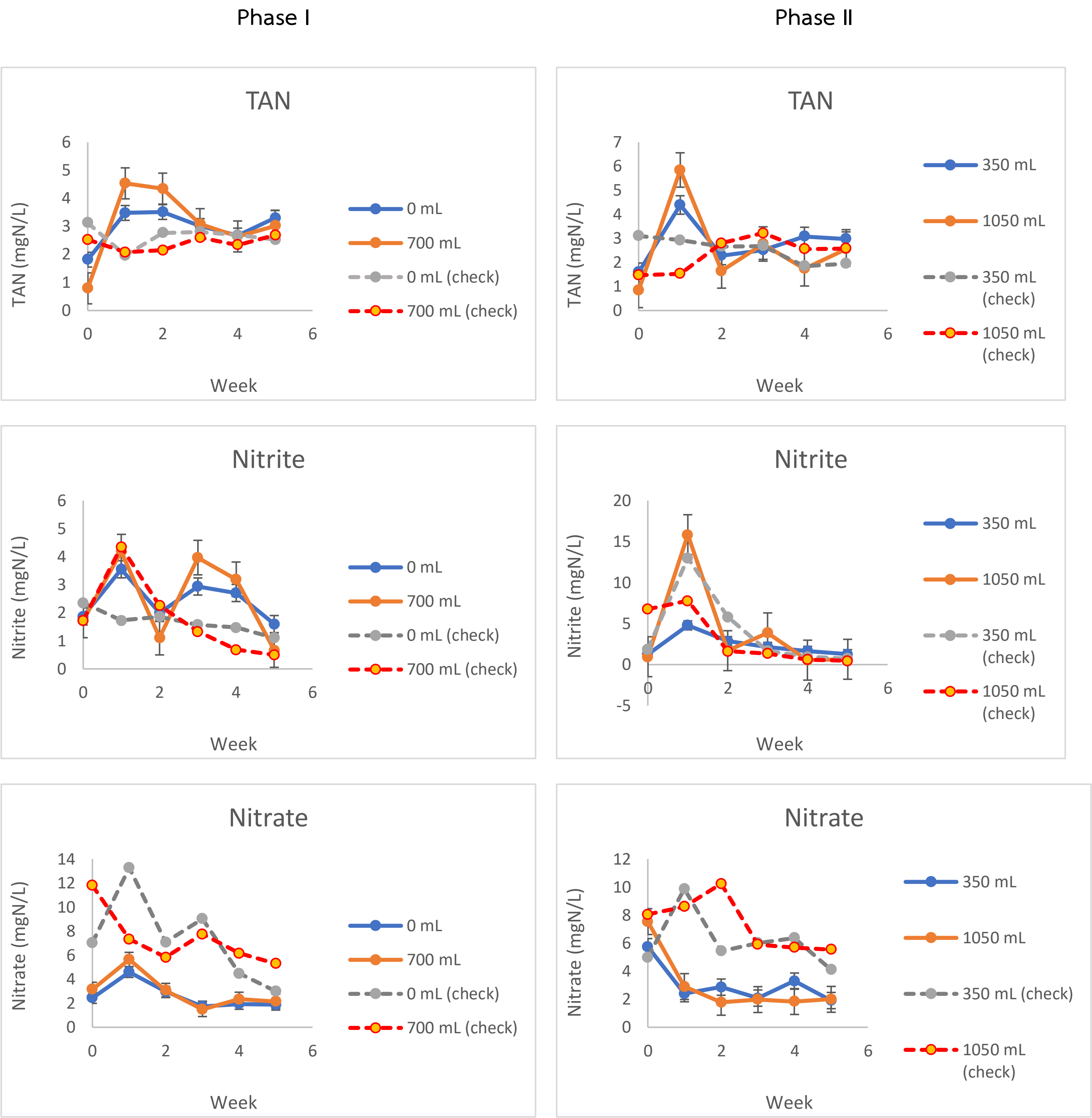


Figure 4.1. Variations of TAN, nitrite, and nitrate with phase1, phase2 and phase3 on bioponic systems. Error bars represent standard deviations of biological replication (n=2)

4.2.1 Total ammonia nitrogen (TAN)

During the first week (phases 1, 2, 3) of cultivation in the bioponic system. TAN (NH_3 + NH_4^+) concentrations from chicken manure compost were oxidized into nitrate and were accumulated in the recirculating water (Figure 4.1). Maximum TAN concentration in each phase reached 3.5, 4.8, 4.5 and 7.7 mgN/L for acetic acid concentration 0 mL, 350 mL, 700 mL, and 1050 mL, respectively. The plants in bioponics used ammonia for growth (Wongkiew, Hu, et al. 2017). This resulted in the reduced ammonia concentration after the maximum concentrations and rebounded and slightly increased from week 4 until the last week of planting (week 5) to 3.3 mgN/L, 2.9 mgN/L and 2.8 mgN/L in acetic acid concentration 0 mL, 700 mL and 1050 mL, respectively. However, TAN concentration of acetic acid concentration 350 mL continued to reach steady state until the last week of planting to 3.5 mgN/L. This was different from acetic acid concentration 0 mL, 700 mL and 1050 mL, which could be because some organic nitrogen compounds took about 4 weeks to mineralize to TAN, and there could be some variations of microbial degradations of TAN and organic nitrogen in the bioponics. The overall trend of acetic acid concentration 0 mL, 350 mL, 700 mL, and 1050 mL were similar to each other for week 0 to 1 (increase in TAN concentration), and the ammonia was detected at the highest concentrations in week 1. This can be explained by the slow growth and adaptation of bacteria in the bioponic system, resulting in a slow ammonia decomposition and rise of ammonia concentration during early week. Nitrifying bacteria (probably in the chicken manure compost) have a relatively slow growth rate, which commonly requires several weeks to establish colonies. It can also be observed that a gradual decrease in ammonia has occurred since week 4 due to the activity of ammonia oxidizing bacteria (Chahal et al. 2016).

4.2.2 Nitrite (NO_2^-)

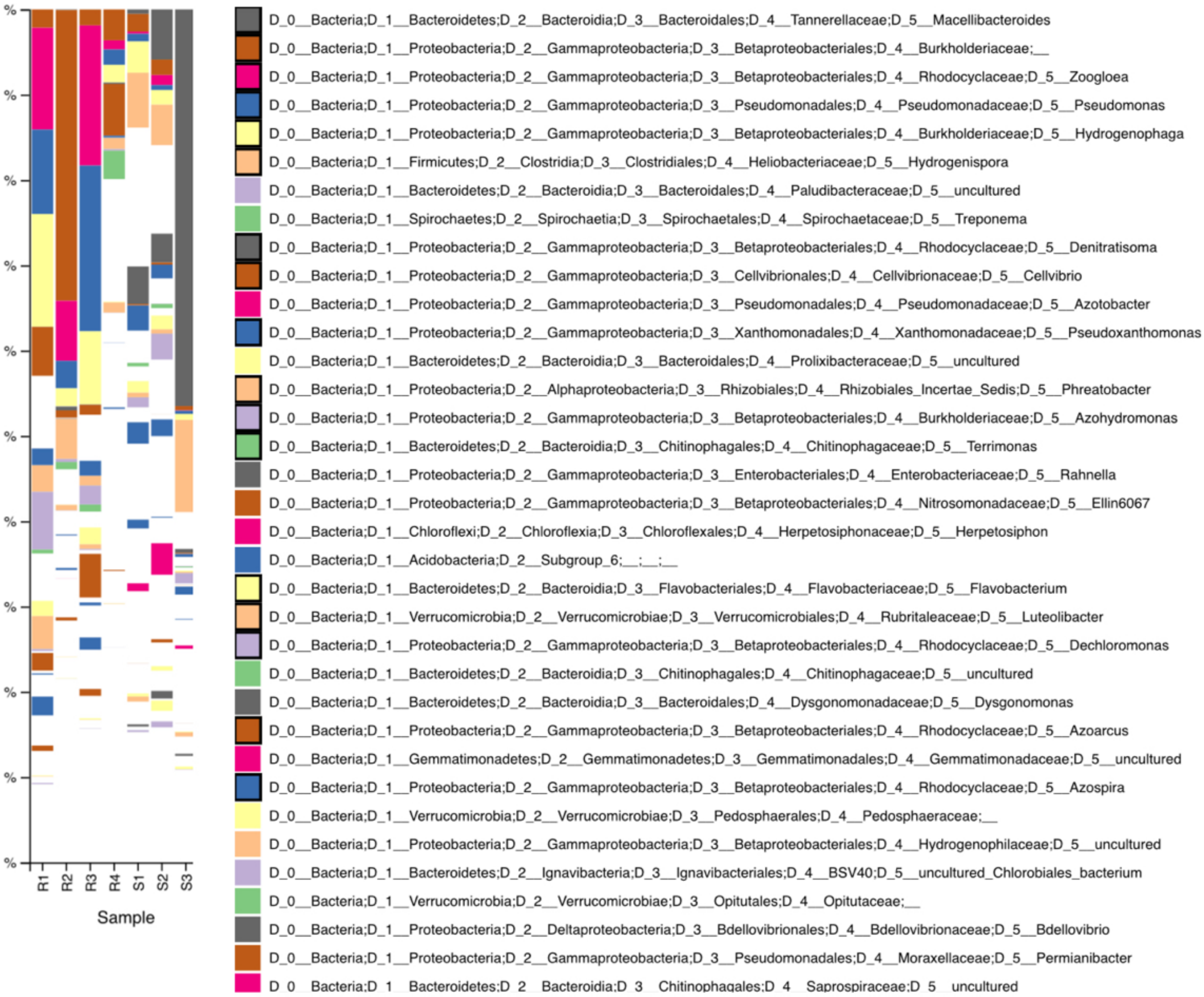
During the first week (phases 1, 2, 3) of cultivation in the bioponics system, the maximum nitrite concentrations (all found in week 1) were 3.3, 5.3, 4.2, and 15.8 mgN/L under acetic acid concentration 0 mL, 350 mL, 700 mL, and 1050 mL respectively, resulting in the nitrite decreasing continuously until the last week of harvest. The minimum nitrite concentrations in acetic acid concentration 0 mL and 350 mL were found at 1.5 and 1.2 mgN/L, respectively. However, nitrite concentration of acetic acid concentration decreased quickly from week 3 - 4 to the last week of planting (week 5), which is different from acetic acid concentration 0 mL and 350 mL. Lower nitrite concentrations supported that plants provide root surface area to nitrifying bacteria for efficient nutrient utilization (Wu et al. 2021)

4.2.3 Nitrate (NO_3^-)

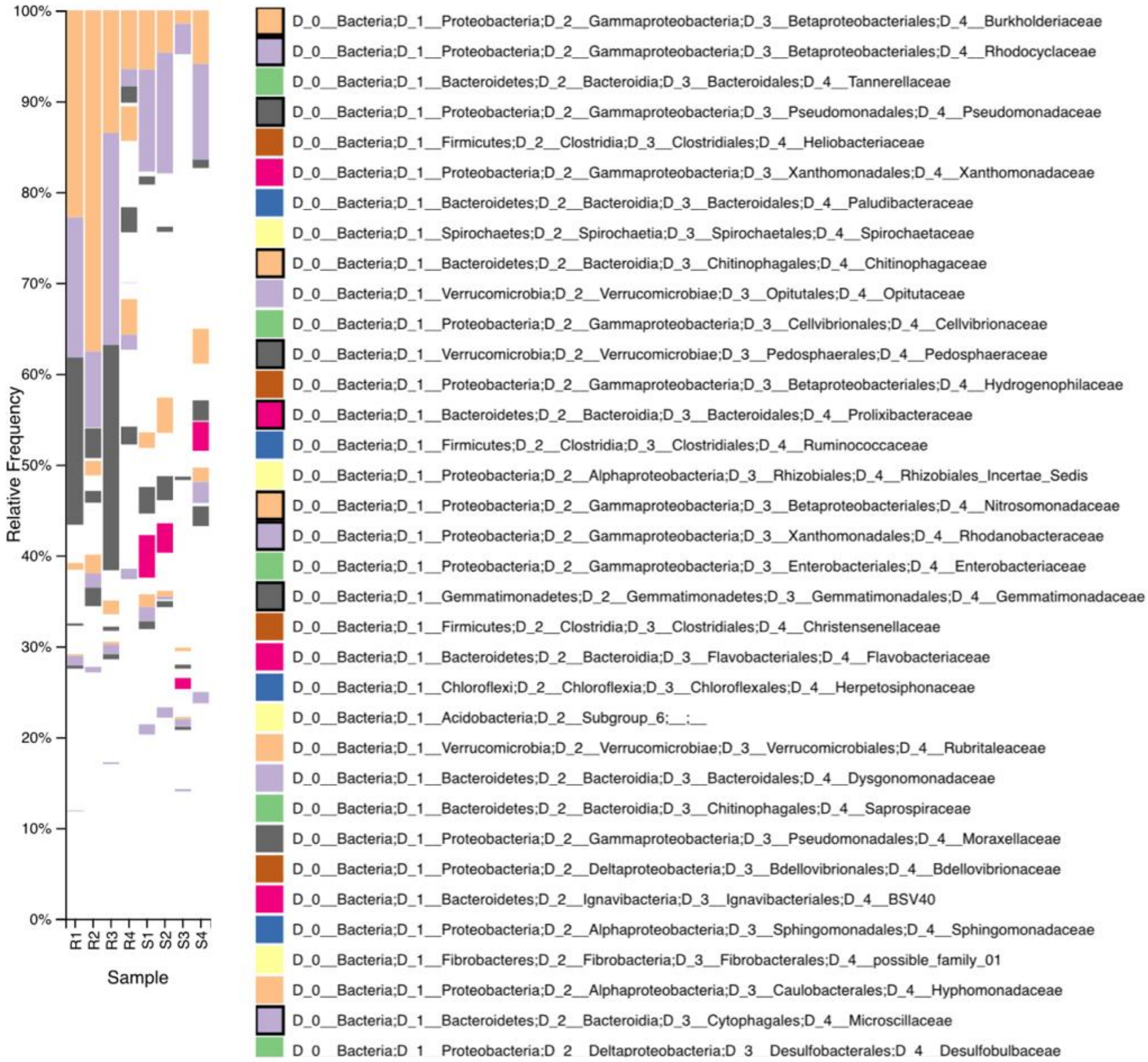
During the first two weeks (phases 1, 2, 3) of cultivation in bioponics. Nitrate concentration in recirculating water increased over the entire operating time while the Cos lettuce continued to grow in the bioponic systems. From Figure 4.1, nitrate concentrations in the system increased up to 4.6, 9.8, 5.6 and 10.2 mgN/L under acetic acid concentration 0 mL, 350 mL, 700 mL, and 1050 mL, respectively, pointing out that microorganisms used nitrate to grow, but still acclimatized in the system (Wongkiew, Hu, et al. 2017). However, due to high nitrate uptake rate, nitrate concentrations decreased continuously from week 3 - 4 to the last week of planting with residual nitrate of 1.8, 4.1, 2.2 and 5.5 mgN/L under acetic acid concentration 0 mL, 350 mL, 700 mL, and 1050 mL respectively. As a result, the imbalance between nitrate generation under acetic acid additions and nitrate utilization by plants can be identified by the occurrence of nitrate accumulation.

4.3. Bacterial community in bioponic systems

Plant roots and sediment (chicken manure) after planting harbored varieties and specific of microbial communities. Microbial diversities in the roots and sediment shared some similarity and difference in bioponic systems under acetic acid addition (concentration 0 mL, 350 mL, 700 mL, and 1050 mL) as shown in Figure 4.2. Linking to the nitrogen transformation, the microbial diversity suggested there were some connection among nitrogen transformations, unit components, and large groups of microorganisms in the bioponic systems.



(a)



(b)

Figure 4.2. Taxonomic affiliation at family level of the bioponic system (sediment and plant roots). Comparisons were made by grouping indicated by a,b

This study found the varieties of bacterial families. The dominant bacterial families in the plant roots were *Burkholderiaceae*, *Rhodocyclaceae*, *Pseudomonadaceae*, *Zoogloea*, *Pseudomonas* and *Hydrogenophaga*. Percent abundances are shown in Table 4.2. The dominant bacterial families in sediment were *Prolixibacteraceae*, *Burkholderiaceae*,*Rhodocyclaceae* and *Macellibacteroides*. *Prolixibacteraceae* were dominant family in sediment but not found in plant roots (Figure 4.2 (b)) of the bioponic system.

Other research, such as aquaponic systems, has found that the diversity of different microbial communities is present, which is different from this bioponic study. This is because the nutrients and substrate were taken from different organic water sources, i.e. in this bioponics system, chicken manure compost was used while the other aquaponic soilless organic study system used the effluent from aquaculture. In addition, *Nitrospira spp.* as the dominant species, which were also found in aquaponics system (S. Wongkiew, Park, Chandran, & Khanal, 2018), but the bioponics found only a very small amount in this study (in systems control = 2.1 – 3.9 %, in system under acetic acid addition = 0.2-0.3%). It can be seen that when acid was added to the system, abundance percentage of bacteria that is important to the system was reduced. Therefore, when compared to relative abundance percentage of other bacteria in the bioponic system, *Nitrospira sp.* was considered to be of very low in abundance, but it was a significant bacteria genus (S. Wongkiew et al., 2018).

4.4. Linking nitrogen transformations to microbial community

The microbial community compositions in bioponics with the Cos lettuce (*Lactuca sativa*) showed high relative abundances of *Nitrosomonadaceae* (1.6 – 3.9 %), which were found in both plant roots and sediment of chicken manure. This microbial family is reported as nitrifying bacteria, one of a small group of aerobic bacteria (family *Nitrobacteraceae*) that use inorganic chemicals as an energy source. They are microorganisms that are important in the nitrogen cycle as converters of soil ammonia to nitrates, compounds usable by plants. The nitrification process requires the mediation of two distinct groups: bacteria that convert ammonia to nitrites (*Nitrosomonas, Nitrosospira, Nitrosococcus,* and *Nitrosolobus*) and bacteria that convert nitrites (toxic to plants) to nitrates (*Nitrobacter, Nitrospina,* and *Nitrococcus*) (M. Z. Wang et al. 2017). Bacteria were found to closely related to some types of denitrifying bacteria *Phreatobacter* (1.3-4.8 % abundance under control conditions, 1.1 – 3.1 % abundance the acid conditions) (H. Wu et al. 2021), *Rhodocyclaceae* (1.9-8.4 % abundance under control conditions, 15.4-23.3 % abundance under acid conditions) (Kämpfer et al. 2005) and *Azohydromonas* (0.2-0.3 % abundance under control conditions, 2.2 – 6.8 % abundance acid conditions) (Xie and Yokota 2005) had significant involvement nitrogen cycling in bioponics, with family involved in nitrate denitrification through to N_2 (Kämpfer et al. 2005). More roles related to nitrogen metabolisms and observed environments reported by other studies were in Table 4.2 – 4.3

Several studies also showed that the microorganisms found in this bioponics existed in other biological systems. For example, *Rhodocyclaceae* was important and related to ammonium oxidizer organisms, was found in sewage treatment plants, rivers and plant root (Kämpfer et al. 2005). The *Nitrosomonadaceae* was found in pig manure and animal compost (M. Z. Wang et al. 2017). It is gram-negative microbe which might include the members of the genus *Nitrosomonas* that oxidizes ammonium ion into nitrite via nitrification called oxidizing ammonia and important in the nitrogen cycle (Hao and Xiao 2017). In addition, *Azohydromonas* was found in different habitats including rice and wastewater. This family are anaerobes capable of fermentative of metabolism (Zhang et al., 2020) and it was found in the recycled water (Wong et al., 2019), suggesting that there could be use nitrogen for their cell metabolisms or nitrogen-fixation and hydrogen-oxidization (Xie and Yokota 2005).

In summary, nitrogen transformation with microbial community in this study suggest that nitrogen is the most important inorganic nutrient for plants and microorganisms. Ammonia (TAN = NH_3 and NH_4) oxidation followed by nitrite oxidation (nitrification) is the process that transforms the TAN into nitrates, which is the form of nitrogen that the plants can uptake. The first reaction is oxidation of TAN to nitrite by ammonia oxidizing bacteria (AOB) represented by members of *Betaproteobacteria* (one of the phyla *Proteobacteria*) and *Gammaproteobacteria*. *(Proteobacteria* phylum) (Abed et al. 2020). The microbes responsible in ammonia oxidation were *Lentimicrobium* (Abed et al. 2020), and *Rhodobacteraceae* (Kämpfer et al. 2005). The second reaction is oxidation of nitrite (NO_2^-) to nitrate by nitrite-oxidizing bacteria (NOB). In the bioponics, *Nitrosomonadaceae* and *Proteobacteria* were found. Denitrification, which contribute to nitrogen loss (nitrate to nitrogen gas) process was carried out by bacteria in the bioponics such as were *Denitratisoma* (Luo et al. 2020) and *Microscillaceae* (Anderson et al. 2011) that change nitrate to dinitrogen gas. Furthermore, within these bioponics, *Xanthobacteraceae* bacteria could fix N_2 to TAN again called N_2 fixation process (Wongkiew, Hu, et al. 2017).

Table 4.2

Taxonomic affiliation at family level of the bioponic system (plant roots).

Name	Level	% abundance		Sources	Role/Environment	References
		control	acid			
Burkholderiaceae	Family	6.5 – 37.6 %	13.5–22.7 %	Drinking water	Nitrogen fixation degrading toluene	(Huang et al. 2014)
Rhodocyclaceae	Family	1.9 – 8.4 %	15.4 –23.3%	Sewage treatment plants, rivers, and plant roots	Plant-associated nitrogen related to ammonium oxidizer organisms	(Kämpfer et al. 2005)
Pseudomonadaceae	Family	1.8 – 3.3 %	18.4 –24.8%	Closed circuit water systems	Creating a microbial biofilm layer on pipe and heat exchanger surfaces, causing a	(Finkmann et al. 2000)

				Drinking water	reduction in efficiency and flow restrictions. Sulphate Reducing Bacteria (SRBs)’s metabolite to produce sulfide under clumps of bacteria	
Chitinophagaceae	Family	1.6 – 3.8 %	0.7 – 1.5 %	Grassland soil	Degrading complex organic matters, such as chitin and cellulose, and showing β-glucosidase activity	(D. Wang et al. 2019)
Pedosphaeraceae	Family	1.3 – 2.7 %	0.3 – 0.4 %	Soil and root surface	Exists in the micro- bial flora in soil and rivers Play an important role in sediments Resistant to environmental changes and/or exogenous invasions, allowing them to	(Salah et al. 2018)

					maintain their community structure.	
Nitrosomonadaceae	Family	2.1 – 3.9 %	0.2 – 0.3 %	Pig manure	Control over nitrification by oxidizing ammonia process could effectively treat the mature leachate total nitrogen removal efficiency	(Wang et al. 2017)
Rhodanobacteraceae	Family	1.5 – 1.6 %	1.1 %	The initial biofilm Marine brown alga	Information of flagella and biofilms, motility, and environmental adaptation.	(Coates et al. 2001)
Gemmatimonadaceae	Family	1.9 – 2.0 %	0.3 – 0.6 %	Sediment	Wastewater treatment Landfill leachate treatment Absorption of heavy metals	(Kreke and Cypionka 1992)

Burkholderiaceae	Genus	3.6 – 34 %	1.9 – 2.2 %	Soil, water, plants, fungi	Key players degrading toluene by para ring hydroxylation	(Yang et al. 2015)
Zoogloea	Genus	1.1 – 7.0 %	12.0 -16.0 %	Fresh water	Wastewater treatment Formation of activated sludge flocs Heavy metal removal	(Linfang Zhang, Fu, and Zhang 2019)
Pseudomonas	Genus	1.8 – 3.2 %	9.9 – 19.4 %	Terrestrial, freshwater and marine	Biofilm formation	(S. Wang et al. 2020)
Hydrogenophaga	Genus	2.1 %	8.6 – 13.2 %	Activated sludge	Wastewater treatment	
Cellvibrio	Genus	0.9 – 6.1 %	1.2 – 5.7 %	Soil bacterium	Ability to degrade plant cell wall Carbon cycling	(Hao and Xiao 2017)

Phreatobacter	Genus	1.3 – 4.8 %	1.1 – 3.1 %	Ultrapure water Drinking water	Nitrogen removal	(H. Wu et al. 2021)
Azohydromonas	Genus	0.2 – 0.3 %	2.2 – 6.8 %	Rice wastewater	Nitrogen-fixing and hydrogen-oxidizing	(Xie and Yokota 2005)
Terrimonas	Genus	0.9 – 3.4 %	0.5 – 0.8 %	Freshwater Polluted farmland soil	Flocculating ability Wastewater treatment	(Huang et al. 2014)
Flavobacterium	Genus	0.1 %	1.8 – 2.0 %	Feeds	Enabling the bacterium to attach to a biotic or abiotic surface and to form biofilms.	(H. Wu et al. 2021)

Luteolibacter	Genus	0.6 – 1.2 %	0.5 – 3.8 %	Soil Sediment	Decomposition of organic matter, remineralization of nutrients, and biogeochemical cycling Removal Chromium	(H. Wu et al. 2021)
Azoarcus	Genus	~ 0	2.1 – 5.1 %	Roots	Nitrogen-fixing	(Simon et al. 2017)
Azospira	Genus	0.1 %	0.1 – 0.4 %	Manure	Wastewater treatment Nitrogen transformation	(Hao and Xiao 2017)

Table 4.3

Taxonomic affiliation at family level of the bioponic system (sediment).

Name	Level	% abundance		Sources	Role/Environment	References
		control	acid			
Prolixibacteraceae	Family	3.2 – 3.3 %	1.2 – 4.7 %	Sediment	Wastewater treatment Nitrogen-fixing Degradation of various organic carbons	(Hao and Xiao 2017)
Microscillaceae	Family	1.1 – 1.2 %	0.2 – 1.1 %	Rice straw composting	Wastewater treatment Nitrification, denitrification Convert NH_4^+ and other reduced nitrogen compounds to nitrogen gas and the gaseous nitrogen oxide	(Anderson et al. 2011)

Burkholderiaceae	Family	4.6 – 5.9 %	1.5 – 6.5 %	Drinking water	Degradation of various organic carbons Degrading toluene	(Xu et al. 2017)
Rhodocyclaceae	Family	10.5 – 13.3 %	3.3 – 11.2%	Sewage treatment plants, rivers and plant roots	Plant-associated nitrogen Related to ammonium oxidizer organisms	(Chahal et al. 2016)
Macellibacteroides	Genus	5.9 %	46.5 %	Groundwater	Wastewater treatment Carbon sources and biofilm carriers	(Lan et al. 2020)
Hydrogenophaga	Genus	1.7 – 2.7 %	0.7 – 3.7 %	Activated sludge	Wastewater treatment Hydrogen-Oxidizing	(Lei Zhang et al. 2019)

Hydrogenispora	Genus	4.8 – 7.7 %	6.4 – 10.8%	Compost	Wastewater Treatment Nitrogen Source Stabilization	(Y. Wang et al. 2019)
Denitratisoma	Genus	3.4 – 7.0 %	0.5 – 4.4 %	Wetlands	Nitrogen Removal and Nitrous Oxide Emission Denitrification Convert NH_4^+ and other reduced nitrogen compounds to nitrogen gas and the gaseous nitrogen oxide	(Luo et al. 2020)
Pseudoxanthomonas	Genus	1.6 – 2.7 %	0.3 – 2.9 %	Sediment	Reduced nitrite	(Simon et al. 2017)
Terrimonas	Genus	0.5 – 2.6 %	0.1 – 0.4 %	Freshwater	Flocculating ability	(Lünsmann et al. 2016)

				Polluted farmland soil	wastewater treatment	
Dechloromonas	Genus	1.7 – 3.0 %	1.2 %	Soil microbe	Denitrification nitrate reduction	(Norberg and Persson 1983)
Azospira	Genus	0.8 – 2.0 %	0.9 – 2.5 %	Sediment	Nitrogen transformation Perchlorate- and nitrate-reducing	(Spiers, Buckling, and Rainey 2000)
Arenimonas	Genus	0.1 – 0.5 %	0.1 – 1.0 %	Compost	Denitrification system Enhanced nitrogen removal	(Kämpfer et al. 2005)
Desulfobulbus	Genus	3.7 %	0.3 – 0.9 %	Freshwater	Sulfate accumulation	(Renouard et al. 2017)

Azonexus	Genus	0.3 – 0.4%	~0.0	Freshwater	Nitrogen fixation	(H. Wu et al. 2021)
Lacunisphaera	Genus	0.5 – 0.8 %	0.1 – 0.3 %	Freshwater	Nitrate reduction to ammonium	(Xie and Yokota 2005)
Aquimonas	Genus	0.7 %	0.5 – 0.6 %	Wastewater	Nitrogen Cycling	(Roveto, Gupta, and Schuler 2021)
Desulfurispora	Genus	0.2 – 0.9 %	0.2 – 0.3 %	River Sediment	Anaerobic biodegradation	(Basson, Flemming, and Chenia 2008)

Lentimicrobium (sp.)	Genus	1.2 %	0.1 – 0.3 %	Landfill leachate	Wastewater treatment Sulfate and nitrogen removal Denitrification system Removal of ammonium	(Abed et al. 2020)
Inhella (sp.)	Genus	0.4 – 0.7 %	0.1 – 0.2 %	Spring water	Wastewater treatment nitrogen removal	(X. Wu et al. 2020)

CHAPTER 5

RESEARCH CONCLUSIONS

5.1. Conclusions

Bioponics showed a high potential for nitrogen recovery under acetic acid addition via nitrate reduction and nitrogen assimilation into organic vegetables (plants) from TAN, nitrite, and nitrate in the bioponics. The highest nitrogen use efficiency (NUE) was found at no acetic acid added (phase 1) in which plants showed highest wet weight biomass yield from all 3 phases. More importantly, TAN (3.0 ± 0.6 mgN/L), nitrite (2.4 ± 0.7 mgN/L), and nitrate (2.6 ± 1.0 mgN/L) were found in bioponics with no acid addition (control). However, under acetic acid adding 1050 mL had higher nitrite and nitrate concentrations than no acetic acid added (3.9 ± 1.9, 3.0 ± 2.2 respectively) in bioponic system. TAN, nitrite, and nitrate assimilated by plants, assisting by and microbes that were fully developed in the systems. This study found *Xanthobacteraceae* bacteria that could fix N_2 to TAN, which plants can take up for growth. It was found that sediment and plant roots of the bioponics were *Nitrosomonadaceae* and *Proteobacteria* that can change nitrite (NO_2^-) to nitrate (NO_3^-) (nitrification process). Plant roots bioponics showed high abundances of ammonia-oxidizing bacteria (*Lentimicrobium* and *Rhodobacteraceae*) and denitrifying bacteria (*Denitratisoma* and *Microscillaceae*). Sediment in bioponic systems shared the microbial genus of *Lacunisphaera* (ammonia oxidation). In addition, bioponic systems were in good performance similar other organic soilless systems such as aquaponic system.

5.2 Research Suggestions

1. Bioponic systems should be studied in various amounts of acid concentration loading to find the acid concentration loading that does not inhibit plant growth.

2. Bioponic systems with pathogenic bacteria should be studied to ensure that the vegetables in the bioponic system are sterile and safe to consumed as raw vegetables.

3. Bioponic systems should be studied in aspect of techno-economics ,to study cost and benefits of such investment project for further real investment, and to study the sensibility analysis of bioponic vegetable business.

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RESEARCHER PROFILE

Name:	Piyatida Hussanodart
Date of birth:	23 October 1998
Place of birth:	Nakhonsawan province
E-mail:	piyatida8991@gmail.com
Graduation:	Bachelor of Environmental Science, Chulalongkorn University Bangkok, Thailand