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SENIOR PROJECT

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Faculty of Science, Chulalongkorn University

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Evaluation of phosphorus and COD concentrations in bioponics under different acetic acid loading rates

By

Benchamart Khumsub

A Senior Project Submitted in Partial Fulfillment of the Requirements for
the Bachelor's Degree of Science Program in Environmental Science
Department of Environmental Science
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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.

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หัวข้อ	การประเมินความเข้มข้นของฟอสฟอรัสและซีโอดีในระบบไบโอโพนิกส์ ภายใต้อัตราการเติมกรดอะซิติกที่แตกต่างกัน
โดย	นางสาว เบญจมาส ขุมทรัพย์ รหัสประจำตัวนิสิต 6033323923
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บทคัดย่อ

ระบบไบโอโพนิกส์เป็นการใช้ของสารอินทรีย์ร่วมกับการปลูกผักแบบระบบไฮโดรโพนิกส์เพื่อเป็นการนำสารอาหารในปุ๋ยอินทรีย์กลับมาใช้ใหม่ทดแทนปุ๋ยอนินทรีย์ที่ใช้ในระบบไฮโดรโพนิกส์ ในระบบไบโอโพนิกส์มีจุลินทรีย์ที่สามารถเปลี่ยนสารอินทรีย์ให้อยู่ในรูปที่พืชสามารถใช้งานได้ ในการศึกษาครั้งนี้เราใช้ Next-Generation Sequencing ในการดูกลุ่มประชากรของแบคทีเรียและศึกษาเกี่ยวกับความสัมพันธ์ระหว่างกลุ่มของแบคทีเรียกับความเข้มข้นของฟอสฟอรัสและซีโอดีโดยการปลูกผักกรีนคอส (Cos lettuce, 14 ต้น/ระบบ) ในระบบไบโอโพนิกส์ และมีการเติมกรดอะซิติกลงไปในระบบ (0 มล., 350 มล., 700 มล., 1050 มล.) จากการทดลองทั้งหมดพบว่าปริมาณการเติมกรดอะซิติกที่ 0 มล./14 ต้น มีค่าประสิทธิภาพในการใช้ฟอสฟอรัส (3.3%) มากที่สุดและได้ผลผลิตที่มีน้ำหนักมากที่สุด (1194 กรัม/14 ต้น) ปริมาณการเติมกรดอะซิติก (350 มล., 700 มล., 1050 มล.) ไม่เกิดความแตกต่างของความเข้มข้นของฟอสฟอรัสทั้งหมด, ความเข้มข้นของฟอสเฟต, ความเข้มข้นของซีโอดี และการเปลี่ยนรูปของฟอสฟอรัสเกิดขึ้นในระบบไบโอโพนิกส์โดยฟอสฟอรัสอนินทรีย์ช่วยในการเจริญเติบโตของพืชผ่านการเชื่อมโยงของชุมชนจุลินทรีย์ในรากพืชและตะกอน แม้ว่าชุมชนจุลินทรีย์ที่มีประโยชน์ในรากพืชจะแตกต่างจากชุมชนของจุลินทรีย์ในตะกอนแต่จุลินทรีย์หลายชนิดแสดงให้เห็นว่าเกี่ยวข้องกับการย่อยสลายของฟอสฟอรัสอินทรีย์และส่งเสริมการเจริญเติบโตของพืชในระบบไบโอโพนิกส์ที่มีการเติมกรดอะซิติกและไม่เติมกรดอะซิติกจะพบจุลินทรีย์ในปริมาณที่ใกล้เคียงกัน เช่น *Phreatobacter*, *Clostridia* ซึ่งบ่งชี้ว่าทั้งสองระบบมีชุมชนจุลินทรีย์ที่คล้ายคลึงกัน จากผลการศึกษาครั้งนี้ทำให้สามารถนำไปพัฒนาประสิทธิภาพของระบบไบโอโพนิกส์ที่ใช้ในการปลูกผักกรีนคอสได้

คำสำคัญ: กรดอะซิติก, กลุ่มจุลินทรีย์, ซีโอดี, ระบบไบโอโพนิกส์, ฟอสฟอรัส

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Abstract

Bioponic system is the use of organic substances in combination with hydroponic vegetable cultivation that reuses of nutrients in organic fertilizers to replace inorganic fertilizers used in hydroponic systems. There are microorganisms that can convert organic matter into usable forms of plants. In this study, we used Next-Generation Sequencing to look at bacterial communities and study the relationship between bacterial groups and phosphorus and COD concentrations by growing lettuce (Cos lettuce, 14 plants/system) in the bioponic system and acetic acid was added to the system (0 ml., 350 ml., 700 ml., 1050 ml.). The efficiency of phosphorus utilization (3.3%) was the highest and the heaviest yield was obtained at no acetic added condition (1194 g / 14 plants). Acetic acid addition (350 ml., 700 ml., 1050 ml.) did not make a difference in total phosphorus concentration, phosphate concentration, COD concentration and the transformation of phosphorus occurred in bioponic systems, with inorganic phosphorus contributing to plant growth through the linkage of microbial communities in plant roots and sediments. Although beneficial microbial communities in plant roots differed from those of sediment microbial communities, many microbes have been shown to be involved in the degradation of organic phosphorus and promote plant growth in bioponic systems. Those with acetic acid added and without acetic acid were found to have similar amounts of microorganisms (*Phreatobacter*, *Clostridia*). This indicates that the two systems have similar microbial communities. From the results of this study, it is possible to develop the efficiency of the bioponic system used in growing cos lettuce by avoiding high acetic acid developed/input to the bioponic systems.

Keywords: Acetic Acid, Bioponics system, COD concentrations, Microbiome, Phosphorus

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CHAPTER 1

INTRODUCTION

1.1 Introduction

The growth of population today has increased in number, which has the same effect in all countries around the world. As the population increases significantly, food and resources will not be sufficient for the needs, causing shortages in some developing countries (Godfray et al., 2010). In the same time, development of various technologies such as intensive agriculture with chemical applications may cause food to get contaminated and damage the environment (Hu et al., 2017). The agriculture and food productions can over utilize important resources and cause the degradation of the natural environment. Agriculture industries release waste into various places causing pollution which is harmful to the world population, environment, and the global climate increases. Therefore, it is needed to find innovations that can save space, environment, and benefit our lifestyles, such as growing edible vegetables using bioponic systems that are environmentally friendly process (FAO, 2012).

Bioponics is the cultivation of plants without soil but using water with dissolved plant nutrients or growing plants in a nutrient solution derived from organic wastes. Nutrients in bioponics are generated by the microbial decomposition of nutrient-rich organic residues that release nutrients in the forms that plants can assimilates. Bioponics is a new way of growing plants especially growing vegetables as food, and bioponics takes advantages of saving space and no contaminations of various toxic chemicals; thus, providing clean vegetables as food (Danaher et al., 2013). Bioponics has two main benefits. The first is to provide a more controlled environment for plant growth; thus, nutrients can be controlled and there is no wastewater discharged to the environments. The second is that plants grown by bioponics circulate organic waste to food that allow the concept of circular economy and applicable in every area. Moreover, bioponics will be more profitable for farmers as it does not require chemical fertilizers added, but it transforms waste into nutrients for plant growths (Mowa et al., 2018). Due to no soil planting, the plants are free of soil disease, weeds, and soil management. Also, plants can be grown very close to each other. As a result, the plant produces a much higher yield while using limited space. In addition, when compared with traditional agriculture, there is very little water used because the container is used, and water is circulated in a confined volume. Bioponic is 100% organic that do not use chemical fertilizers, pesticides or herbicides (Fang and Chung 2018).

Phosphorus is the primary macronutrient for plants because phosphorus is a component of nucleic acids and a main element of DNA and RNA required for protein synthesis (Maathuis, 2009). Furthermore, phosphorus is a component of phospholipids of cell membrane (Hawkesford et al., 2012). Phosphorus used in agricultural systems can be applied for crop productions using either mineral fertilizers from phosphate rock or organic waste compost (Syers, Johnston, & Curtin, 2008). Thus, organic fertilizer from animal manure could be used extensively as organic waste compost to recovery nutrients such as nitrogen and phosphorus in agriculture (Mullins, 2009). Chicken manure has high concentrations of phosphorus, nitrogen and soil organic carbon that are essential for plants growth in organic farming (Ravindran et al., 2017).

There are several microbial genera responsible in phosphorus transformations in biological processes, which can occur as in bioponics. There are several enzymes produced from microorganisms to utilizable organophosphate such as nucleosidase (to produce phosphate DNA or RNA), carbon-phosphorus (C-P) lyase (to breakdown the C-P bond), and phosphatases (can be classified as alkaline phosphatase and acid phosphatase). The sources and concentrations of phosphorus are the major factors that shift the microbial communities. Inorganic phosphorus is also significant for microorganism because it is the major source to get utilized by microorganism (Zheng et al., 2019). Phosphorus solubilizing microorganisms are the groups of microorganisms that can solubilize precipitated inorganic phosphorus (Richardson & Simpson, 2011) to soluble phosphate that is the bioavailable phosphorus form for plant use (Alori, Glick, & Babalola, 2017). Phosphorus solubilizing microorganisms can also enhance plant growth, phosphorus accumulation, and production of chlorophyll content in plants (da Silva Cerozi & Fitzsimmons, 2016b). *Bacillus*, *Pseudomonas* and *Enterobacter* are the main groups of phosphorus solubilizing bacteria (Khan, Jilani, Akhtar, Naqvi, & Rasheed, 2009). In addition, low inorganic phosphorus concentration decreases removal efficiencies of chemical oxygen demand (COD) by heterotrophic bacteria (Zheng et al., 2019).

For growing plants in the bioponic system, pH is important to control nutrients availability of the solution in the system. Since bioponics generates nutrients from organic residues, organic carbon, organic phosphorus, and phosphate are the main bioindicator to evaluate the performances of bioponic systems in term of nutrient generations (Atkin et al. 2004). The appropriate level pH level also increases in phosphorus generation and organic degradation rates. If it is unable to maintain the pH level suitable for growing plants, plants will unable to have and absorb sufficient nutrients in the system to take full advantage. The pH of the solution should generally be in the range of 5.5 - 6.5, or best in the range 5.8 - 6.2, which is a good range for plants to absorb all the nutrients (Domingues et al. 2012).

On the other hand, if the pH is higher than 7 for 2 - 3 days in a row, it will cause abnormal absorption of phosphorus, iron and manganese because the nutrients can precipitate in the water. When plant grows progressively, pH level in water can increase. This is because in vegetative growth, plants are mostly absorbed by phosphate (PO_4^{3-}) (absorbing ions). Therefore, bicarbonate (HCO_3^-) radicals are released in the same amount, increasing the pH of the solution. In bioponic cultivation, pH is measured and adjusted to suit the growth of plants. In this study acetic acid will be added to the system to increase phosphorus availability for plants and to stimulate organic degradations and phosphorus solubilization in bioponics. Acetic acid is an organic acid with the chemical formula CH_3COOH.

The importance of pH is also related to the functions of various microorganisms. Normal organic substances can cause root rot in plants. With microorganisms degrading nutrient-rich organic materials, the organic materials will release various nutrients such as phosphorus. Microorganisms digest and makes manure decay then releasing nutrients that are beneficial to plants. Organic compost in bioponics affects the growth of plants more slowly than chemical fertilizers because organic compost needs the microorganisms to decompose first. Then, plants can absorb nutrients. There are various microorganisms to decompose manure and various organic substances in bioponic systems. Microorganism work fully and efficiently in bioponic systems when the pH is appropriate which could be done by adding acetic acids (Fujiwara et al.2012). Manure and organic matter are decayed very slowly, release nutrient slowly, and benefit plants. However, there are few studies in the bioponic system.

In this study, organic acids will be added to the system, particularly acetic acid, to investigate the linkage between microbial communities and phosphorus transformations under acid conditions to improve phosphorus utilization efficiency and increase crop yields. The study could provide a a bioponic techniques for farmers interested in biodiversity and provide a new approach to the biological and circular economy. Thus, it is needed to study on the phosphorus and COD concentrations in bioponic systems under different acetic acid additions, and comparison of microbial community under acetic acid addition and without acetic acid additions.

1.2 Research Objectives

1.) To evaluate phosphorus and COD concentrations in chicken manure-based bioponic systems under different acetic acid additions.

2.) To compare microbial community between bioponics with acetic acid additions and without acetic acid addition in term of organic degradations and phosphorus solubilization.

1.3 Expected Outcomes

1.) Understanding the characteristics of phosphorus and COD concentrations in chicken-manure based bioponics, which can be further applied for managing phosphorus availability and plant growth.

2.) Understanding of key microbes that are associated with acetic acid addition, phosphorus utilization, and organic degradation.

CHAPTER 2

LITERATURE REVIEWS

2.1 Wastewater treatment using plants

Phytoremediation, the application of vegetation and microorganisms for recovery of nutrients and decontamination of the environment, has emerged as a low-cost, eco-friendly, and sustainable approach compared to traditional biological and physicochemical processes. Livestock wastewater is one of the most severe pollution sources to the environment and water resources. When properly handled, livestock wastewater could be an important alternative water resource in water-scarce regions (Hu et al. 2020). Liquid organic fertilizers produced from agricultural residues and industrial wastes are becoming increasingly popular. These fertilizers are produced by simple fermentation processes using organic wastes as carbon substrates. Liquid organic fertilizers consist of essential plant nutrients and beneficial microorganisms, which recycle organic matter. Microorganisms have an important role on the degradation of substrates in the fermentation process. The removal of contaminants in the constructed wetland systems is related to the function of the biological process that is formed when wastewater passes through plant roots, while the plants assist in the purification process. At the plant roots, an oxygen rhizosphere is created, while in other parts of the layer there are anaerobic zones and poorly oxygenated ones. According to Birkedal et al. (1993), the plant roots and rhizomes allow to keep proper hydraulic conductivity of the layer and cause the looseness of its internal structure. The system of developed roots and rhizomes allows an intensive development and growth of the plants on the soil-plant layers, which can have a direct impact on high transpiration of water from the plant surface and show a significant reduction of the amount of sewage flowing out of the systems, and even the disappearance of the drain (Gregersen and Brix, 2001). The existing research results show that constructed wetlands and phytoremediation can be used not only for highly efficient wastewater treatment, but at the same time in order to produce biomass for energy purposes (Cerbin et al., 2012, Posadas et al., 2014).

2.2 Phosphorus recovery from waste

The prospect of decreasing the availability of phosphorus ores in years have posed a threat to the world's agricultural systems (Cordell et al., 2009, Cordell et al., 2011, Schröder et al., 2011). The introduction of P recycling and P use reduction can substantially improve the longevity of the natural phosphorus reserves (Koppelaar and Weikard, 2013). It is necessary to restructure agricultural practices to close the P cycle for an adequate P management in a changing world (Sharpley et al., 2015). Closing the P loop in the agricultural sector requires a system capable of managing phosphorus with a high level of flexibility. Phosphorus has to be delivered at the right place and time, i.e., where and when plants need phosphorus the most, with no excessive environmental and economic costs (Bateman et al., 2011). Such management includes practices to recover phosphorus in usable forms from places in the food system where nutrients usually concentrate (wastewater treatment plants, livestock production facilities, compost operations, and food processing plants) and recycle it through crop production (Yorgey, 2016).

2.3 Effects pHs on phosphorus transformations

Solution pH in aquaponics systems is a compromise between microbial and plant demands. 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 aquaponics systems is managed near pH 7.0 (Wortman, 2015). A recent study determined that pH 6.0 was optimal for plant growth and nitrogen utilization efficiency in bioponics at the expense of increased N_2O emission due to high denitrification (Zou et al., 2016). The forms in which phosphorus exists in solution also changes according to pH. The pKa for the dissociation of H_3PO_4 into $H_2PO_4^-$ and then into HPO_4^{2-} are 2.1 and 7.2, respectively (Schachtman et al., 1998). Therefore, in the pH range maintained in typical biosystems, phosphorus is mostly present in the form $H_2PO_4^-$, while H_3PO_4 and HPO_4^{2-}have lower activities. Plants can only absorb P as the free orthophosphate ions $H_2PO_4^-$ and HPO_4^{2-} (Becquer et al., 2014). The rate of phosphate uptake decreases as the pH of the external solution increases, which is explained by a reduction in the concentration of $H_2PO_4^-$, which is the substrate of the proton-coupled phosphate symporter in the plasma membrane. In the pH range of 5.6–8.5, conversely, a decrease in pH can increase the activity of proton-coupled solute transporters and enhance anion uptake (White, 2012). As pH increases above 7.0 in aqueous solutions, most of the dissolved phosphorus reacts with calcium forming calcium phosphates. Gradually, reactions occur in which the dissolved free phosphate species form insoluble compounds that cause phosphate to become unavailable. In alkaline solutions, calcium is the dominant cation that reacts with phosphate (Siebielec et al., 2014). A general sequence of reactions in alkaline solutions involves the formation of dibasic calcium phosphate dihydrate, octocalcium phosphate, and hydroxyapatite. Each phosphate product formation results in the decrease of solubility and availability of phosphate (Siebielec et al., 2014).

2.4 Aquaponic, hydroponic and other similar systems

Hydroponics is the soil-less cultivation of plants using mineral nutrient solutions (Sardare and Admane, 2013). In this method, nutrients are fed directly to the roots. It is a highly precise and demanding method that requires a greater amount of production knowledge, experience, technical skill, and financial investment than many other greenhouse systems. There are many advantages of growing plants under soil-less culture over soil-based culture (Savvas, 2002). Six types of hydroponic systems are recognized including NFT (Nutrient Film Technique), Deep Water Culture (DWC), Wick Hydroponics, Ebb and flow hydroponic systems (also called flood and drain), Drip hydroponics and Aeroponics show in figure1.

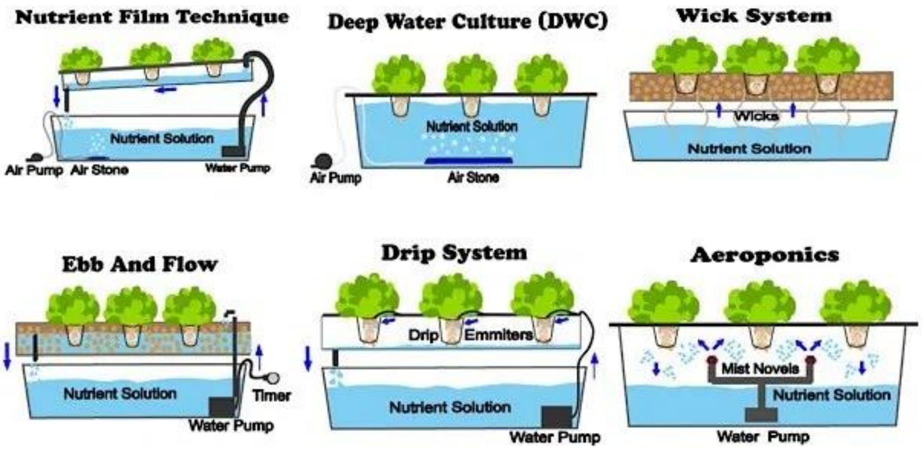

Figure 2.1 Six types of hydroponic systems (Available from: https://www.nosoilsolutions.com/6-different-types-hydroponic-systems/)

Hydroponic lettuce is commonly produced using either the nutrient film technique (NFT) or the floating raft method, both as closed systems. This means less water is used when compared to ground cultivation. While hydroponics is an excellent technique for the cultivation of vegetable crops and other plants, organic fertilizers cannot be used in conventional hydroponic systems, which generally utilize only inorganic fertilizers. The nutrient film technique (NFT) is a system that allows the solution to circulate through the plant roots in a thin film. The solution is constantly circulated, 2-3 mm thick, is very popular in industrial production systems, this system is suitable for winter crops and salad vegetables. Advantages of the NFT system 1. There is no need to have a water regulator because this system is always watering the plants. 2. The system of providing solution to plants is not complicated. 3. It is the system with the most efficient use of water and nutrients. 4. No planting material to be disposed of 5. Able to grow crops continuously throughout the year. Do not waste time preparing the planting system. For example, you can grow salad vegetables up to 8-10 times / year. But this system has some disadvantages: There will be costs for equipment and tool maintenance. Along with the knowledge and understanding of the operation of the system as well the operation of this system is equipped with electrical equipment, so it is suitable for the locality where it is convenient and there are no power outages.

Aquaponics has received considerable attention due to system’s capability to raise fish at high density, sustain adequate water quality, minimize water exchange, and produce a profitable vegetable that is responsible for the direct assimilation of dissolved fish wastes and products of microbial breakdown (Danaher et al., 2013) show in figure 2.

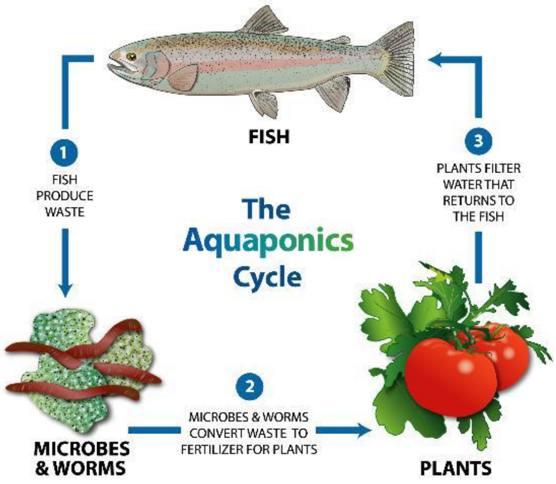

Figure 2.2 Aquaponic systems (Available from: https://www.motherearthnews.com/organic-gardening/aquaponic-gardening-growing-fish-vegetables-together)

Enhanced biological phosphorus removal (EBPR) is a widely adopted technology for treating wastewater including the efficient removal of phosphorus. It addresses the growing concern about the environmental impacts caused by excessive nutrients in water bodies, known as eutrophication. This technology is based on alternating anaerobic and aerobic conditions to encourage the uptake of 'luxury' phosphorus (P) by organisms that thrive under these conditions: polyphosphate accumulating organisms (PAOs). There are many possible variations of the conventional configuration (Anaerobic/Aerobic or A/O), including the addition of one or multiple anoxic stages to achieve denitrification with the simultaneous removal of P (Sedlak, 1991). Denitrification is achieved through the action of multiple groups of heterotrophic microorganisms, including PAOs (DPAOs), who can use nitrogen compounds (nitrate and/or nitrite) as electron acceptors. Theoretically, and as shown in laboratory-scale studies, anoxic phosphorus removal is advantageous since it allows simultaneous P and nitrate removal on the same carbon source, thus reducing oxygen requirements as well as sludge production (Kuba et al.1996a).

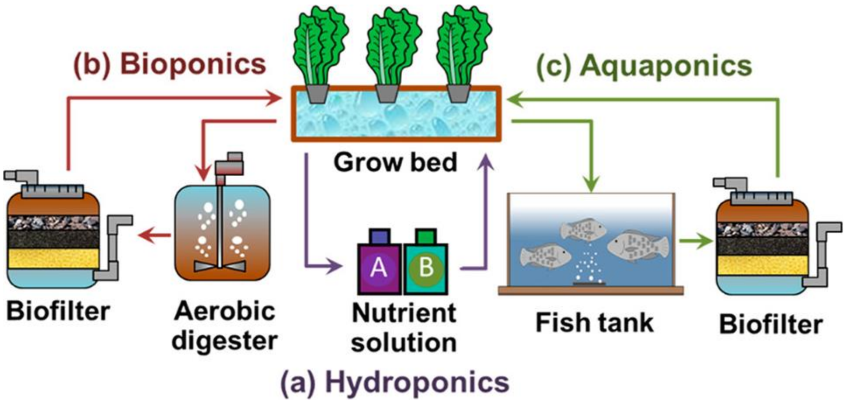

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Figure 2.3 Schematics of hydroponics (a), bioponics (b), and aquaponics (c).
(Available from: https://doi.org/10.1021/acsestengg.0c00196)

2.5 Microbial community

Phosphorus in the sludge is important as not all kinds of phosphorus fraction exhibit bio-availability. Therefore, the phosphorus fraction will be selected to evaluate the performance of the phosphorus release behavior rather than total phosphorus (TP) concentration. It was also reported that 40–60% of reactive P (RP) was existed in sewage sludge, which could be recoverable and available for chemical reactions. Non-reactive P (NRP) in the sludge phase should be firstly converted to soluble-RP (sRP), which could be recovered for direct use. It was known that RP was known as inorganic P (IP), orthophosphate (ortho-P), while NRP was known as organic P (OP) or polyphosphates. Standards in Measurements and Testing (SMT) protocol was used to investigate the distribution of phosphorus fractional phase in sludge samples. Thus, the NRP converted to RP fraction was contributed to the anaerobic P release and recovery. In addition, anaerobic sludge is the pool of microorganisms for P release, which relies on a group of bacteria, i.e., phosphorus accumulating organisms (PAOs). PAOs could store carbon sources as intracellular polymeric (poly-β-hydroxyalkanoate, PHA) and release ortho-P from the microbial cells using the energy of the degradation of glycogen and polyphosphate (poly-P). PAOs are identified as mesophiles or even psychrophiles. When the temperature is higher than 20 °C, PAOs and glycogen accumulating organisms (GAOs) will compete for the carbon source uptake. In such a case, GAOs waste the energy, but no polyphosphate will be accumulated for the P recovery. Moreover, the activity of the phosphatase, which is a kind of hydrolytic enzyme for the P release from organic phosphorus in the sludge, is influenced by the temperature. Therefore, temperature is a key parameter because it affects the microbial population characteristics and the metabolic activity of anaerobic sludge (Zeng et al., 2019).

CHAPTER 3

MATERIALS AND METHODS

3.1 Development of a bioponic system

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 (as is the figure 3). A bioponic system consisted of one recirculating tank for aeration and recirculation water (~18 liters), one up- flow biofilter (~18 liters), and two-channel grow bed (14 plants per one bioponic system). Dry chicken manure was applied in the biofilter and was used as a nutrient source for the systems because chicken manure contains 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. Romaine lettuce is one of the most popular fresh organic vegetables (Demir, 2019). Biochemical filter pad was used to increase surface area for microbial attachment in the up-flow biofilter. The bioponic systems were operated in duplicate (n=2).

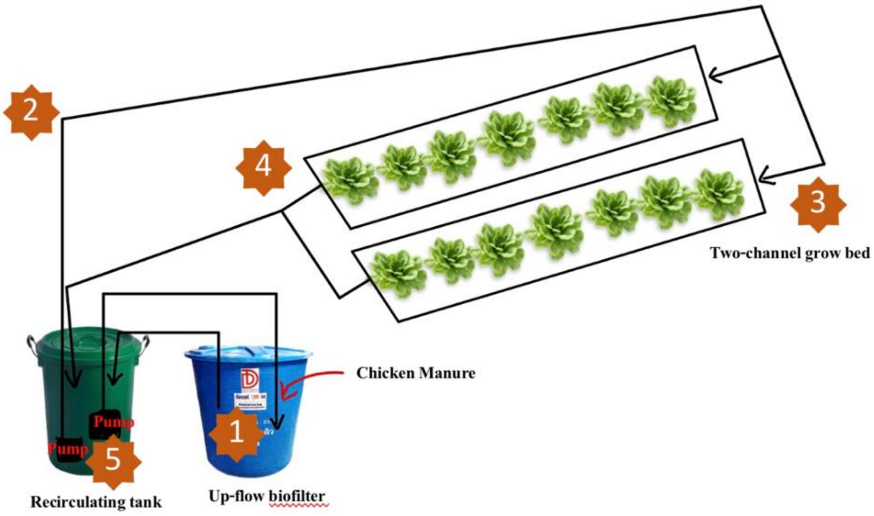

Figure 3.1 Schematic diagram of a bioponic system in this study, consisting of biofilter (1), nutrient feeding line with pump (2), NFT grow bed (3), water outlet back to recirculating tank (4), and biofilter water-feeding pump

3.2 Experimental setup

For this study, there was 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. Each system used 500 grams chicken manure compost per system. After seed germination for about 1 weeks, plants were be transferred to grow bed of bioponics (14 plants per system). Acetic acid (5% w/v) was added every week: phase 1 (0 mL acetic acid vs. 700 mL acetic acid), phase 2 (350 mL acetic acid vs. 1050 acetic acid), and phase 3 (0 mL acetic acid, 350 mL acetic acid, 700 mL acetic acid, 1050 mL acetic acid). Water samples were collected every week. Experiments in phases 1 and 2 were conducted in duplicate (n=2), phase 3 was a cross-check phase (n=1).

3.3 Water samples

Water in bioponics was sampled and was monitored weekly for total phosphorus (TP) concentrations, phosphate (PO_4^{3-}) concentrations, COD concentrations, and pH levels. TP and phosphate were analyzed using 4500-P C. Vanadomolybdophosphoric Acid Colorimetric Method followed by spectrophotometric method (APHA, 2005). COD were analyzed using dichromate followed by 5220 C. Closed Reflux, Titrimetric Method (APHA, 2005). And, pH was monitored using a pH meter. Bioponic systems were operated in duplicate in phases 1 and 2. At the end of each experiment, all plants were harvested and weighed for wet weight and moisture content. And in phase I, root and sediment samples (0 mL acetic acid vs. 700 mL acetic acid) were selected to be analyzed for microorganisms associated with this bioponic system.

Table 3.1 Experimental setup in phases 1, 2, and 3

Phase / Parameters	Phase I	Phase II	Phase III
Chicken manure	500 g	500 g	500 g
Weekly acetic acid content	Concentration 1 = 0 ml (n=2) Concentration 2 = 700 ml (n=2)	Concentration 1 = 350 ml (n=2) Concentration 2 = 1050 ml (n=2)	Phase I + Phase II (no replication)
Every week, pH and DO measurements are performed.	✓	✓	✓
Water samples will be collected every week for COD and total P analysis.	✓	✓	✓
Microbial community analysis	✓	X	X

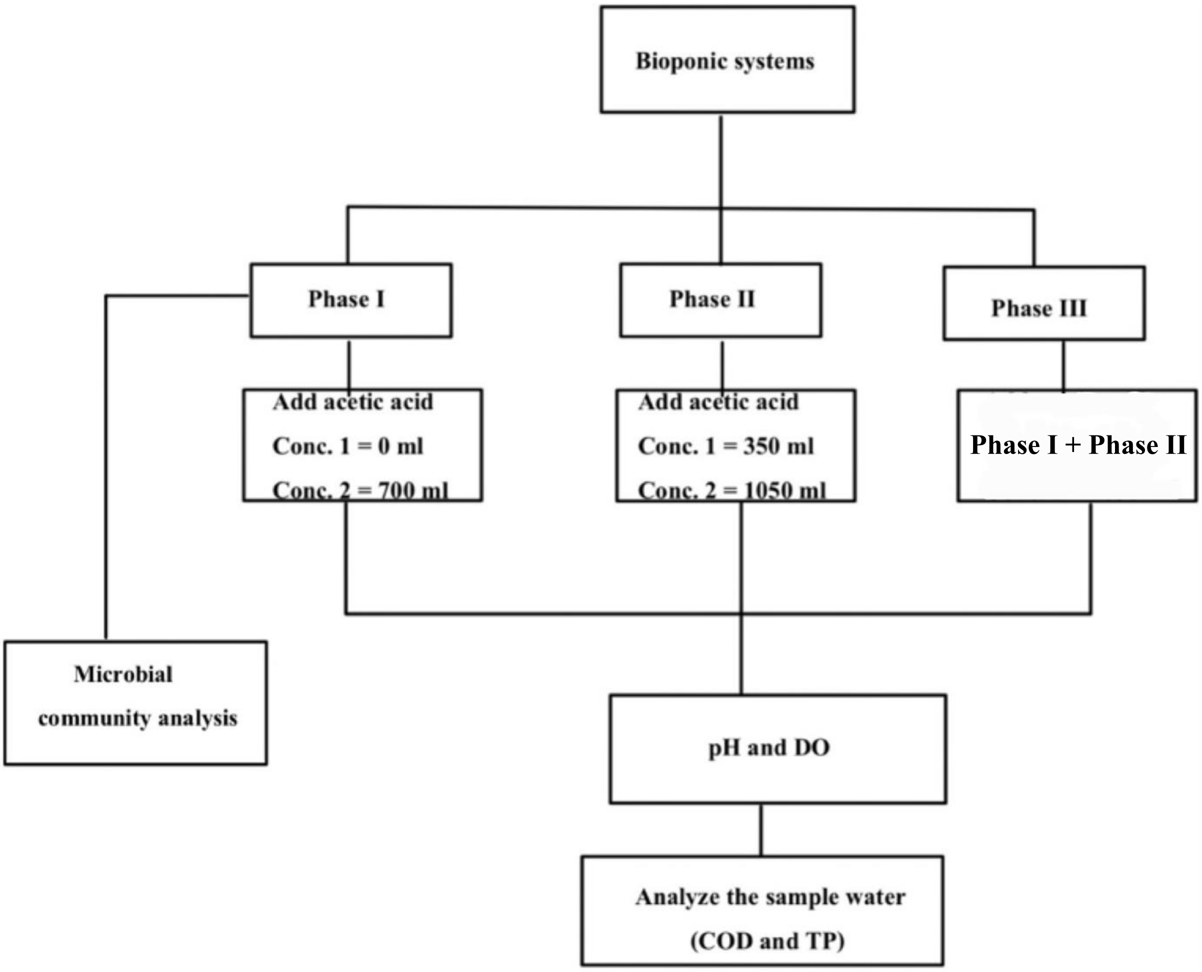

Figure 3.2. Diagram of experimental setup

3.4 Calculation

The mass balance of P in bioponic systems was calculated as follow

$$C_P.M_C = \frac{d}{dt}(C_{PO4\text{-}P})V + \frac{Pplant}{T} + \frac{Psed}{T} + \frac{Pprecp}{T}$$

Phosphorus Use Efficiency (PUE) in bioponic systems was calculated as follow

$$PUE = \frac{Pplant}{Cp.Mc}$$

Where, C_P is the tractions of P in chemical fertilizer solutions (g P/L); M_c is the chemical fertilizer application rate (L/day); T is the production duration (days); Pplant is the average P assimilated by plants at harvest (g P); Psed is the P in solid waste accumulated in biofilter at the end of each trial (g P); $C_{PO4\text{-}P}$ is the concentrations of PO4-P in recirculating water (g P/ L); Pprecp/T is the rate of P loss via organic P precipitation (g P/day) (Cerozi and Fitzsimmons, 2017; Wongkiew et al., 2017); V is the volume of recirculating water (L)

3.5 Microbial analysis

At the end of phase 1, plant root samples will be taken from the bioponic system for microbial analyses. The sample were characterized using Next-Generation Sequencing (NGS) Analysis (Illumina platform) targeting 16S ribosomal ribonucleic acid (rRNA) genes to enable a detailed characterization of overall microbial community in the test bioponic systems. The 16S rRNA gene sequencing (V3-V4 regions) was conducted at Omics Center, Chulalongkorn University using Greengenes as reference databases. The bacterial taxonomy was classified based on Greengenes version 13.8. QIIME 2 was used as a bioinformatic tool.

3.6 Statistical analysis

The results will be statistically tested using t-Test (between two groups, α = 0.05) to compared the amount of DO, temperature, phosphate concentration, total phosphorus concentration, COD concentration, plant dry weight and phosphorus use efficiency in within each phase of the experiment.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Bioponics system performance

The experiment demonstrated the cultivation of Cos lettuce in bioponics systems under different acetic acid levels of 0 ml, 350 ml, 700 ml, and 1050 ml. The TP concentration ranges of acetic acid at 0 ml, 350 ml, 700 ml, and 1050 ml were 34.2-34.7 mgP/L, 44.3-49.3 mgP/L, 39.7-49.3 mgP/L, and 47.3-49.5 mg P/L, respectively. The ranges of phosphate concentrations under different acetic acid levels of 0 ml, 350 ml, 700 ml, and 1050 ml were 22.5-26.9 mgP/L, 29.7-44.4 mgP/L, 25.2-44.8 mgP/L, and 27.3-47.1 mgP/L, respectively. The ranges of COD concentrations in a 0 ml acetic acid level were 251.1-442.7 mg /L, and in the 350 ml, 700 ml, and 1050 ml acid levels were 245.5-400 mg /L. The pH range in the 0 ml acetic acid level was 7.0-8.0, and those in the 350 ml, 700 ml, 1050 ml acid levels were from 5.0 to 6.8. The DO range in the 0 ml acetic acid level was 6.5-6.6 mg /L, and in the 350 ml, 700 ml, 1050 ml acetic acid levels were 5.8-6.9 mg/L. The temperature range in the 0 ml acetic acid level was 29.4-30.6 °C, and in the 350 ml, 700 ml, 1050 ml acetic acid levels were 28.8-30.9 °C. There was no significant difference of COD, TP, and phosphate concentrations (for systems with and without acetic acid addition (phase 1) and for systems with acetic acid additions of 350 and 1050 ml (phase 2)). (Table 4.1).

Table 4.1 Operating parameters (DO concentrations, pH, water temperatures), phosphorus, and COD concentrations in bioponics systems without acetic acid and acetic acid addition.

	Phase 1		**Phase 2**		**Phase 3**			
	0 mL	700 mL	350 mL	1050 mL	0 mL	350 mL	700 mL	1050 mL
TP (mgP/L)	34.7 ± 8.4	39.7 ± 13.8	49.3 ± 24.2	47.3 ± 26.1	34.2 ± 10.7	44.3 ± 17.2	49.3 ± 14.2	49.5 ± 16.5
Phosphate (mgP/L)	22.5 ± 9.0	25.2 ± 9.7	29.7 ± 13.7	27.3 ± 13.5	26.9 ± 14.5	44.4 ± 12.0	44.8 ± 10.2	47.1 ± 11.9
COD (mg/L)	251.1 ± 98.9	245.5 ± 94.6	286.8 ± 173.9	251.8 ± 131.0	442.7 ± 84.5	299.0 ± 39.3	400.0 ± 82.8	368.0 ± 75.0
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

Values reported as mean ± standard deviation (n = 12 for phases 1 and 2, n = 6 for phase 3) comparisons were made within each column. No significant differences ($p > 0.05$) were found in systems with acetic acid added and without acetic acid added.

Table 4.1 illustrates no significant difference in the parameter levels from the three phases of the bioponics system ($p \geq 0.05$) difference in phases 1, 2, and 3. The plants were grown in a similar environment, and the amount of acetic acid added (0 ml, 350 ml, 700 ml, 1050 ml) could make a long-term difference in the growth and water parameters. The phosphate concentrations in the three phases hold no significant difference ($p \geq 0.05$) since the phosphate concentrations were converted from organic phosphorus by microorganisms, similar to total phosphorus concentrations for all three phases. In addition, other studies have utilized vegetable cultivation—lettuce in the aquaponic system with phosphorus concentrations similar to this experiment. The phosphorus content in plants in the study was 0.91% w / w (Cerozi & Fitzsimmons, 2017). A comparison of the mean phosphorus content in this study indicated that the bioponics system was effective compared to other literature, despite adding different amounts of acetic acid. By comparison, the mean values of total phosphorus concentrations (TP) in phase 1 were insignificant ($p \geq 0.05$), differing from phase 2 TP concentrations. Additionally, the concentrations of TP in phase 3 were insignificant. ($p \geq 0.05$), different from TP concentrations in phase 1 and phase 2 could imply that the addition of acetic acid did not cause long-term differences in phosphate concentrations.

Furthermore, COD concentrations and phosphorus concentrations in all three phases of the experiment were not significantly different ($p \geq 0.05$). Therefore, the addition of acetic acid was not different from the COD concentration during period of planting. Other studies assessed the effect of pH on phosphorus availability in the aquaponic system, and pH was found to be effective. The phosphorus transformations and its biologically usable form of phosphorus with the highest transformation rate were reported at pH of 8.5 (da Silva Cerozi & Fitzsimmons, 2016a), so the bioponics system has the optimal pH to convert phosphorus into a biologically usable form.

Table 4.2 Plant yield, phosphorus in a plant, phosphorus in manure, phosphorus remaining, phosphorus dissolved, and PUE in bioponics systems with acetic acid added and without acetic acid added.

	Phase 1		Phase 2		Phase 3			
	0mL	700mL	350mL	1050mL	0mL	350mL	700mL	1050 mL
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
P in plant (gP)	0.4 ± 0.2	0.2 ± 0.1	0.2 ± 0.2	0.2 ± 0.1	0.5	0.3	0.3	0.2
Manure P remaining (g)	9.0 ± 0.1	9.0 ± 0.7	8.7 ± 0.4	9.0 ± 0.7	8.4	8.3	8.7	8.5
P dissolved (gP)	7.0 ± 0.1	6.9 ± 0.7	7.3 ± 0.4	6.9 ± 0.7	7.5	7.6	7.2	7.4
P in manure (gP)	15.9 ± 0.0	15.9 ± 0.0	15.9 ± 0.0	15.9 ± 0.0	15.9	15.9	15.9	15.9
PUE (%)	2.6 ± 1.0	1.1 ± 0.6	1.5 ± 0.9	0.9 ± 0.8	3.3	1.9	1.8	1.5

According to Table 4.2, the weights of plants in the condition without acetic acid ranged from 921.5- 1194.0 g. The weight of the plants with acetic acid volumes, 350 ml, 700 ml, 1050 ml, were 338.0- 696.0 g. Thus, the weight in a system without acetic acid was higher than the acetic acid added. It suggests that adding acetic acid can cause plant deficiency, affecting plant growth. Therefore, the weight of the plants in the acetic acid-added system was lower than that without acetic acid addition although p value >0.05). However, the trend of decrease in plant growth vs. increase in acetic acid dose was obvious and significant (linear regression, $p < 0.05$). Typically, the plant (lettuce) has a rapid growth rate, and the plant weight reached the exponential phase after three weeks after transplanting (Cerozi & Fitzsimmons, 2017). Therefore, days of planting and the amount of acetic acid are essential for plant production. Phosphorus is the component of nucleic acids, DNA, and RNA required for protein synthesis (Maathuis, 2009). Furthermore, phosphorus is the component of phospholipids of the cell membrane. Thus, phosphorus is vital for plant growth (Hawkesford et al., 2012). It can be seen from the Table 4.2 that the phosphorus in plants transformed to the phosphorus in manure. The residual phosphorus in the manure and the dissolution of phosphorus in both systems are the same as the systems without the addition of acetic acid; the addition of acetic acid was not significant affecting the phosphorus concentrations ($p > 0.05$). Moreover, phosphorus efficiency (PUE) in the system without acetic acid is higher than in the acetic acid system. The reason is that the plants' weight without the acetic acid system was higher than that of the acetic acid added system. Therefore, the phosphorus concentration in the system with acetic acid was lower than that of the system without acetic acid. Alternatively, another study uses the *Bacillus* sp. to increase the performance of the aquaponic system. The aquaponic that use *Bacillus* sp. had the PUE (phosphorus in the plant) at 43.6% (da Silva Cerozi & Fitzsimmons, 2016b). The PUE in the aquaponic systems was higher than in this experiment because it contained an amount of phosphorus in the bulk sediment.

4.2 Dynamics of phosphorus and COD concentrations in bioponics

The line graph (Fig. 4.1) illustrates the concentrations of total phosphorus (TP), phosphate, and COD in bioponics systems in phases 1, 2, and 3 with different acetic acid added (0 ml, 350 ml, 700 ml, 1050 ml)

The phosphorus concentrations in bioponics systems can be established through the physicochemical degradation of inorganic nutrients and microbial degradation of phosphorus in manure (Ebid et al., 2007). First, phosphorus release resulted in increased total phosphorus and phosphate concentrations in the bioponics system (Fig. 4.1), and the rate of plant phosphorus utilization gradually decreased, respectively. Organic compounds that release plant nutrients and the use of organic carbon in bioponics systems from microbial degradation represented the same dynamics. The system did not affect the degradation or use of nutrients for plant growth, and it could be a guideline that the addition of acetic acid did not alter the efficiency of the bioponics system.

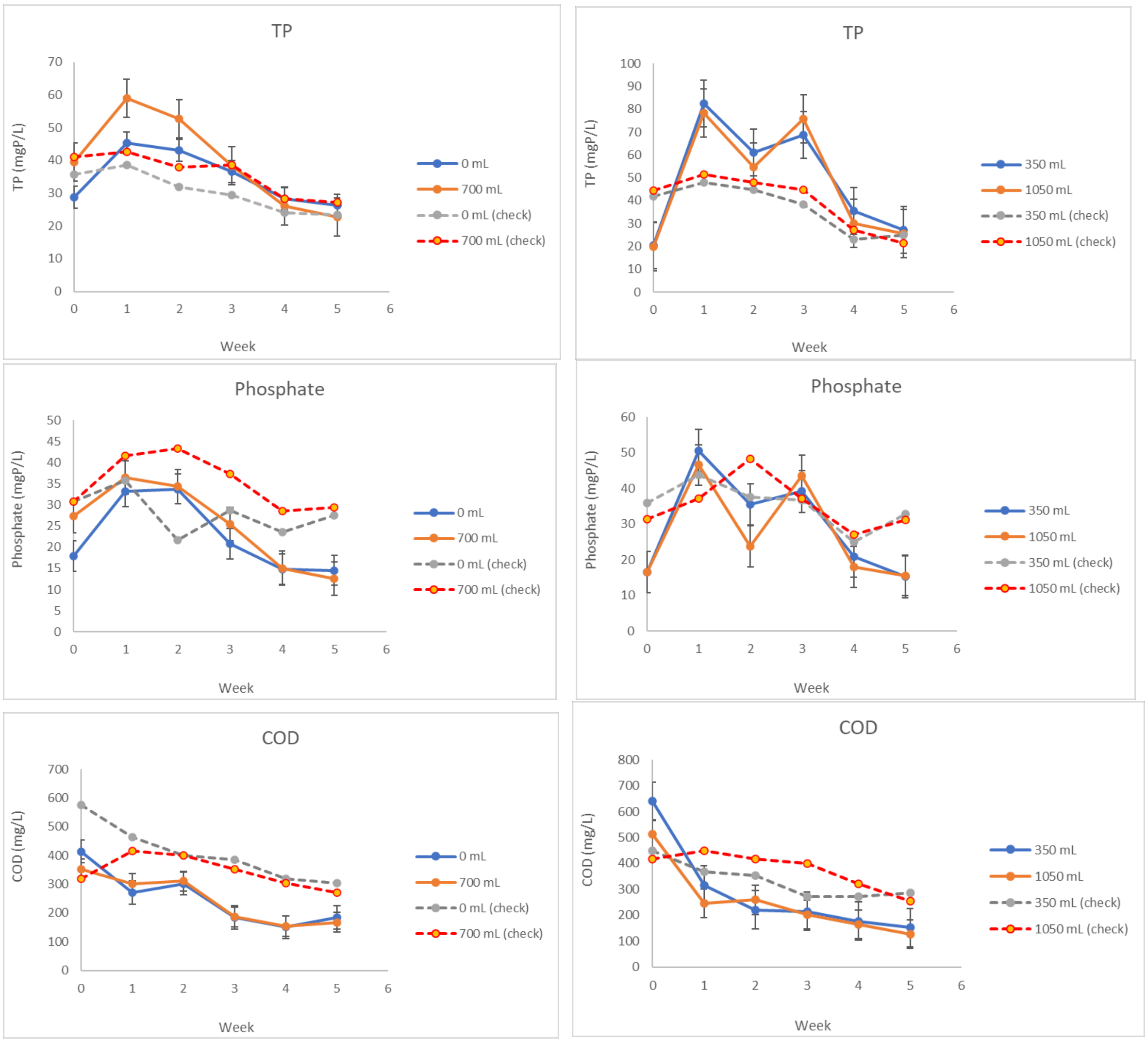

Figure 4.1 The variation of phosphate, phosphorus, COD, and difference of phosphorus and phosphate with different acetic acid addition (0 ml, 350 ml, 700 ml, 1050 ml) in bioponics systems

By comparison, during the first week after transplanting, the COD concentration increased dramatically, and the COD concentration decreased sharply to the end of the phase. However, The COD concentrations in the three phases were not significantly ($p \geq 0.05$) different from each other. The underlying cause might be the amount of acetic acid loading (0 ml, 350 ml, 700 ml, 1050 ml) was not in a high amount that could cause an effect on the COD concentrations.

4.3 Bacterial community in bioponics systems

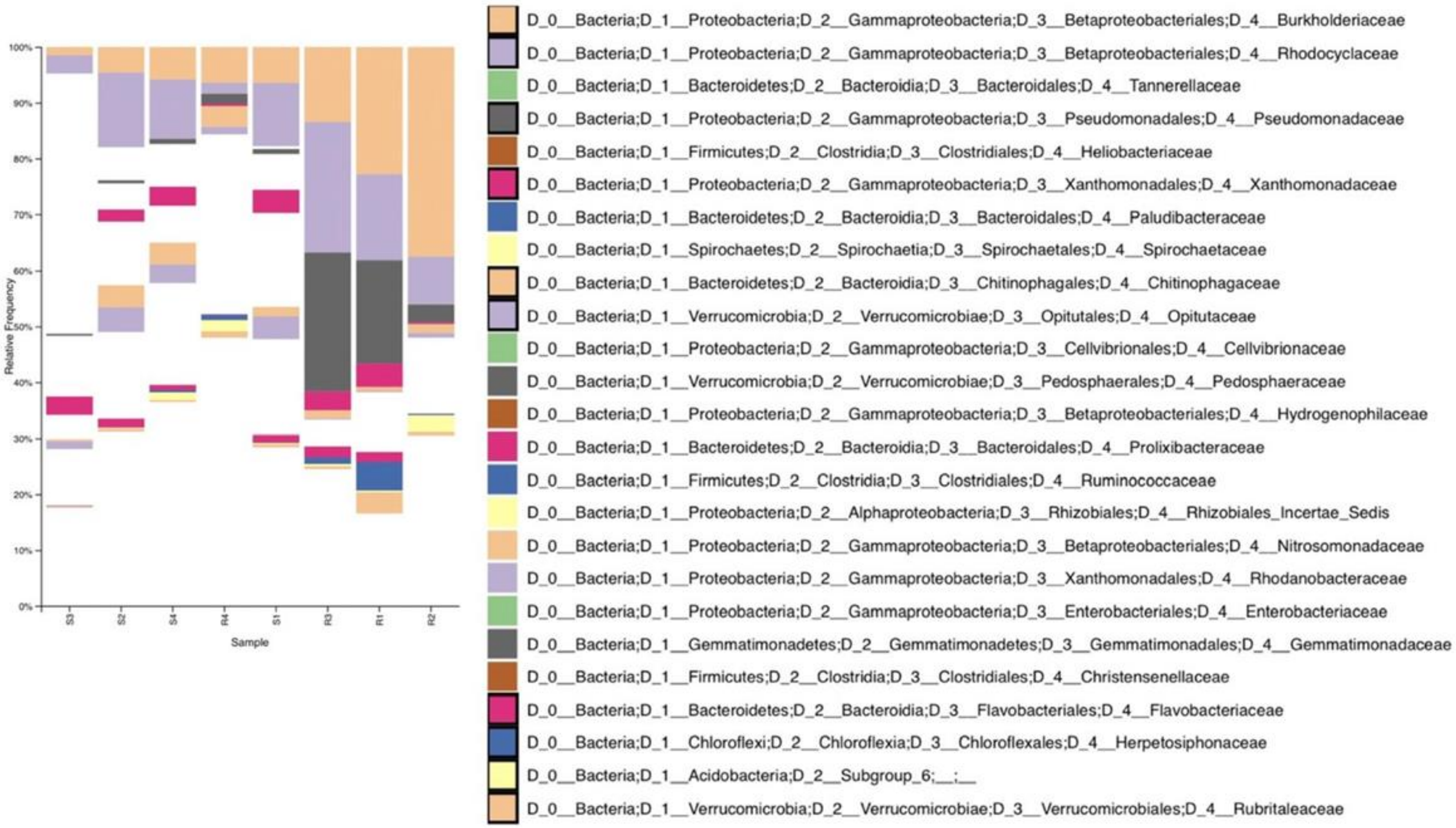

Figure 4.2 Microorganisms found in cos lettuce roots during bioponics systems with acetic acid added and without acetic acid added

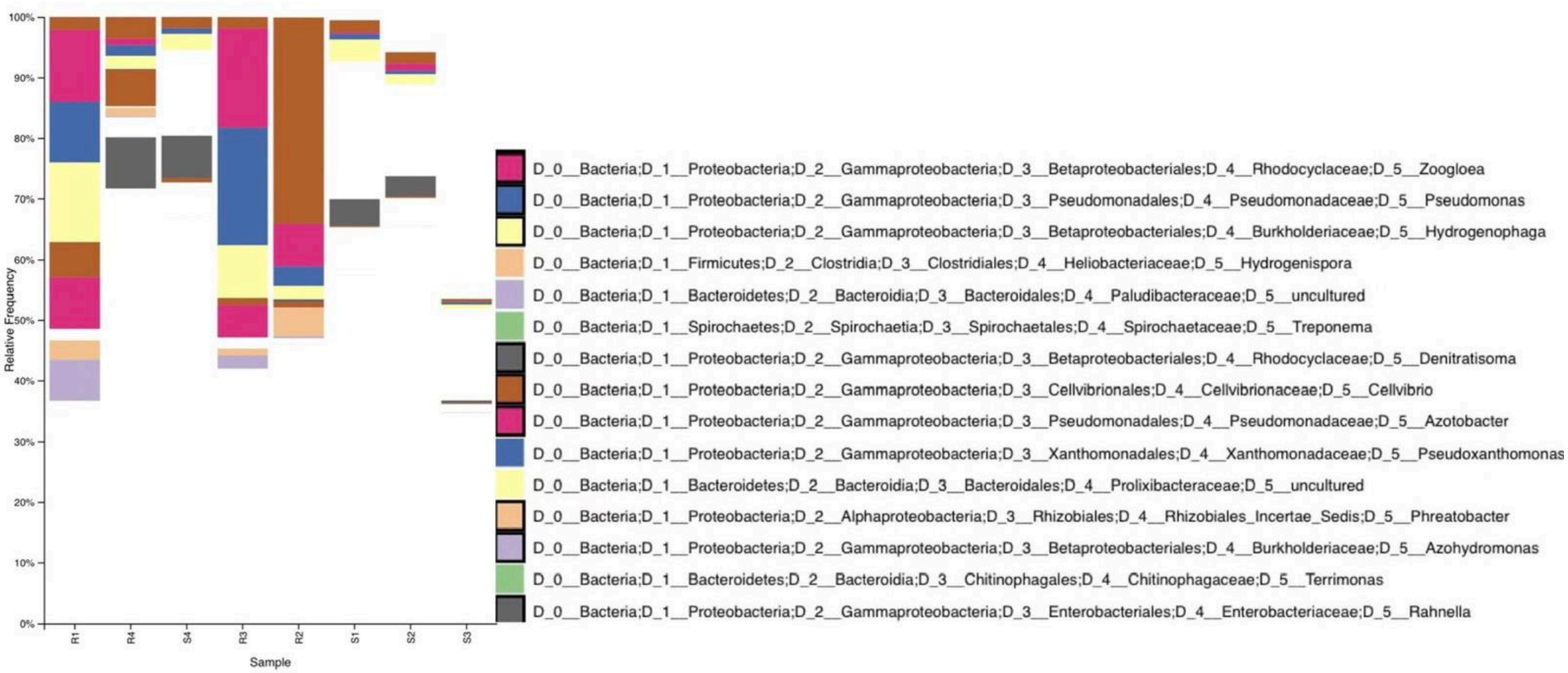

Figure 4.3 Microorganisms found in cos lettuce roots during bioponics systems with acetic acid added and without acetic acid added

Table 4.3 Microorganisms found in cos lettuce roots during bioponics systems with acetic acid added and without acetic acid ad**ded.**

Name	Level	% Abundance		Found at	Role	Reference
		control	acid			
Burkholderiaceae	family	6-38%	13-23%	Root surface or root interior	They are bacteria associated with a wide variety of plants and can adapt to the environment.	(W. C. Chen et al. 2021)
Rhodocyclaceae	family	2-8%	15-23%	Wastewater source	It has a role in phosphorus removal.	(Wang et al. 2021)
Flavobacteriaceae	family	0-0.1%	1-2%	They are bacteria that reside within the cells of plants.	It assists in plant growth.	(Hollants et al. 2013)
Pseudomonadaceae	family	1-3%	18-25%	It can be found everywhere in soil, water, and plants.	It is a pathogenic bacterium that reduces plant growth.	(Mulaosmanovic et al. 2021)
Herpetosiphonaceae	family	0.2-0.9%	1-5%	Found in fertilizers.	It helps in the degradation of potent chitin as a plant disease control agent.	(Hui et al. 2020)
Xanthomonadaceae	family	0.2-0.4%	3-4%	Root surface or root interior	It assists in plant growth.	(W. C. Chen et al. 2021)

Acidobacteria	phylum	3-4%	0.5-1.2%	Root surface or root interior	It assists in plant growth.	(W. C. Chen et al. 2021)
Rubritaleaceae	family	0.6-1%	0.5-4%	Found in plants	It assists in plant growth.	(Kasai et al. 2007)
Chitinophagaceae	family	1-4%	0.7-1.5%	Found in sediment	It decomposes organic matter.	(Hou et al. 2021)
Opitutaceae	family	0.8-1.2%	0.1-0.2%	Found in water resources	It assists in plant growth.	(Tegtmeier et al. 2018)
Azotobacter	genus	0%	5-8%	Found in sediment	Azotobacter is a group of Gram-negative, free-living, nitrogen-fixing aerobic bacteria inhabiting the soil.	(Sumbul et al. 2020)
Betaproteobacteriales	order	20-49%	37-38%	Found in water resources	Had the ability to transformed the phosphorus to the bioavailable.	(Boden, Hutt, and Rae 2017)
Zoogloea	genus	1-7%	12-16%	Found in water resources	It promotes treating wastewater by helping to decompose Organic matter in wastewater.	(Gan et al. 2011)

Pseudomonus	genus	1-3%	10-19%	Found in water resources	It promotes treating wastewater by helping to decompose Organic matter in wastewater.	(Ma et al. 2020)
Hydrogenophaga	genus	2-2.1%	8-13%	Found in water resources	It promotes treating wastewater by helping to decompose Organic matter in wastewater.	(Tegtmeier et al. 2018)
Phreatobacter	genus	1-4%	1-3%	Found in water resources	It is the bacteria that increase the turbidity of the water.	(Sumbul et al. 2020)
Azohydromonas	genus	0.1-0.3%	2-7%	Found in plants	It degrades toxins that may arise from the accumulation of excess phosphorus.	(Zhao et al. 2021)
Rahnella	genus	8-8.4%	0%	Found in plants	It can degrade organophosphorus pesticides.	(Verma and Chatterjee 2021)
Denitratisoma	genus	0.1-0.4%	0%	Found in water resources	It is a microorganism that contributes to biodegradation.	(Qin et al. 2020)
Cellvibrio	genus	0-6%	1-5%	Found in fresh water	It is a microorganism that helps in degradation.	(Kwon et al. 2019)

The bar charts (Fig. 4.2, Fig. 4.3, Fig. 4.4, Fig. 4.5, and Fig. 4.6) show the varieties of microbial communities. The root of lettuce and the compost of sediment after harvesting had the difference and similarity of the microbial diversities. The microbial communities reported in the bar charts had links with the phosphorus transformation and organic degradations in bioponics systems.

Table 4.3 shows that the microorganisms found in the root of cos lettuce in the bioponics system without acetic acid had a lower percentage of the microbes found in the system. Systems contain acetic acids such as *Rhodocyclaceae, Flavobacteriaceae, Pseudomonadaceae, Herpetosiphonaceae, Xanthomonadaceae, Azotobacter, Zoogloea*, and some microorganisms that are overlapping with acetic acid added systems such as *Burkholderiaceae, Rubritaleaceae, Chitinophagaceae, Opitututaceae, Phreatobacter, Cellvibrio* most of these microorganisms affect the growth of plants. Roles of each microbial group on phosphorus and organic degradations and possible habitats are shown in Tables 4.3 and 4.4. Another study on microbial diversity in the aquaponic system reported that the *Proteobacteria* were the dominant phylum of bacteria in the water and the plant roots of the aquaponic system. Interestingly, *Actinobacteria* could transform the phosphorus to the bioavailable only 10% in the water (Schmautz et al., 2017). By comparison, the study found that the dominant bacterial circle in the acetic acid and without acetic acid system was *Burkholderiaceae* and *Betaproteobacteriales*. They are bacteria associated with a wide variety of plants and can adapt to the environment that could transform the phosphorus to them bioavailable. It is apparent that the lettuce root had similar microbial diversity in both systems.

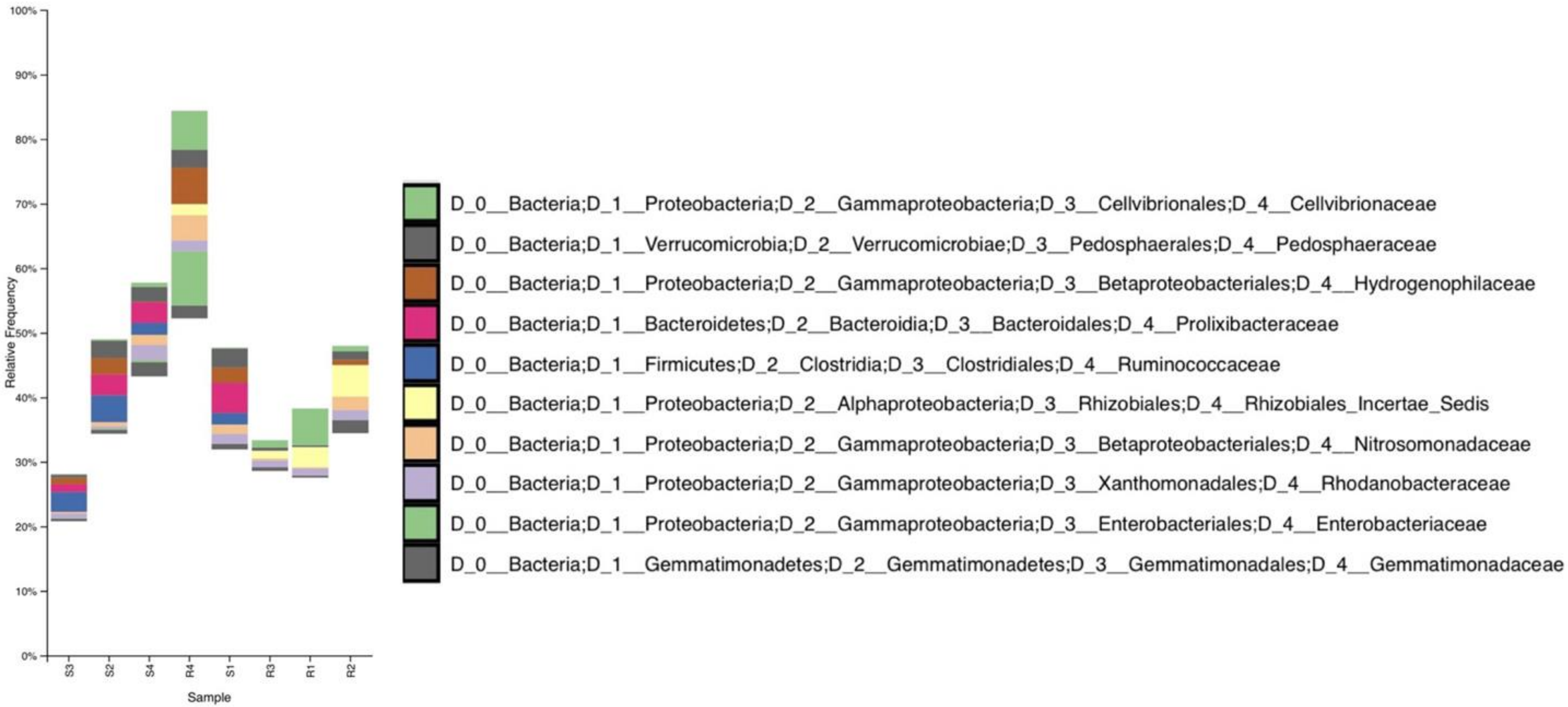

Figure 4.4 Microorganisms found in sediment during bioponics systems with acetic acid added and without acetic acid added (1)

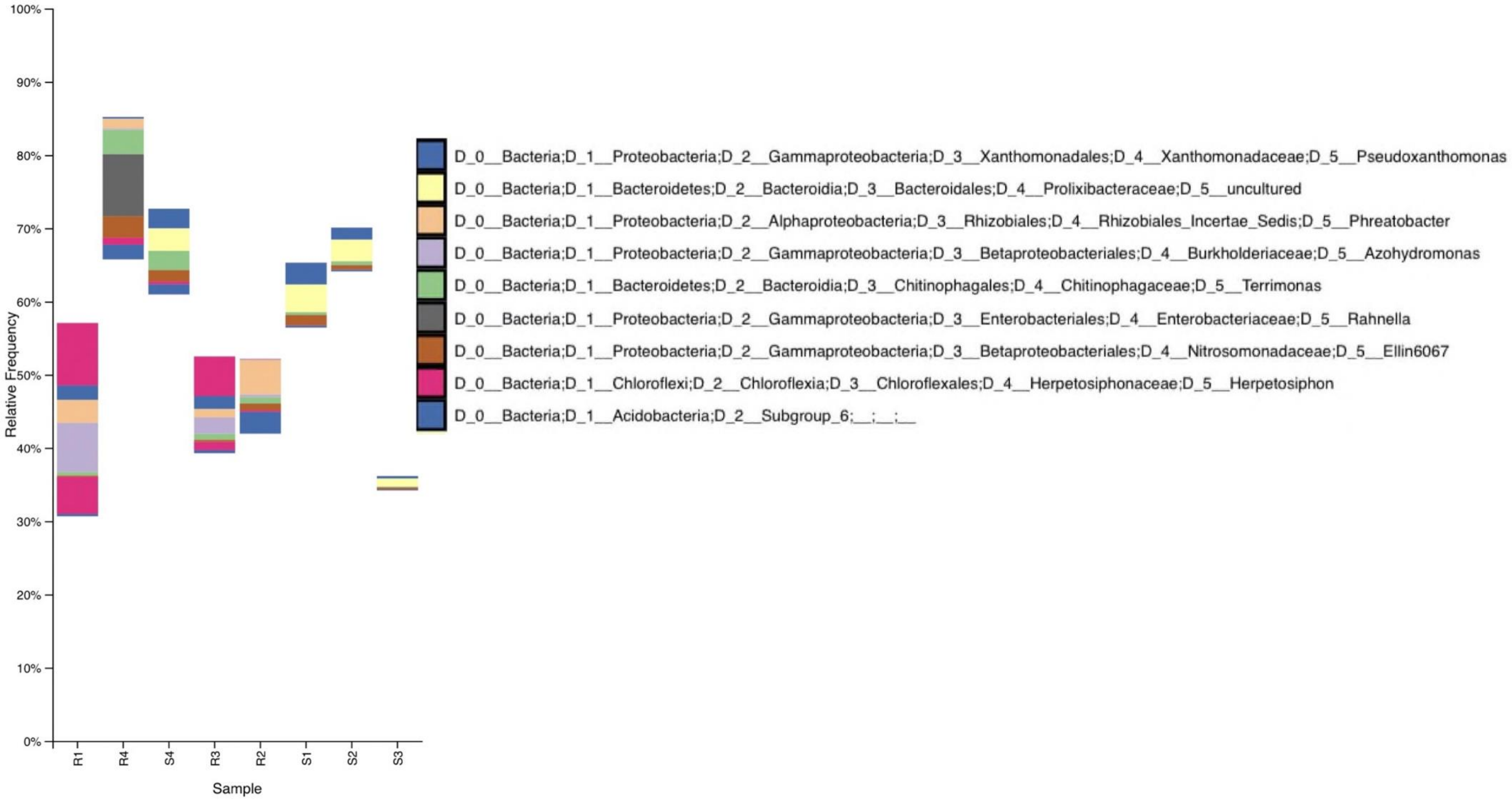

Figure 4.5 Microorganisms found in sediment during bioponics systems with acetic acid added and without acetic acid added (2)

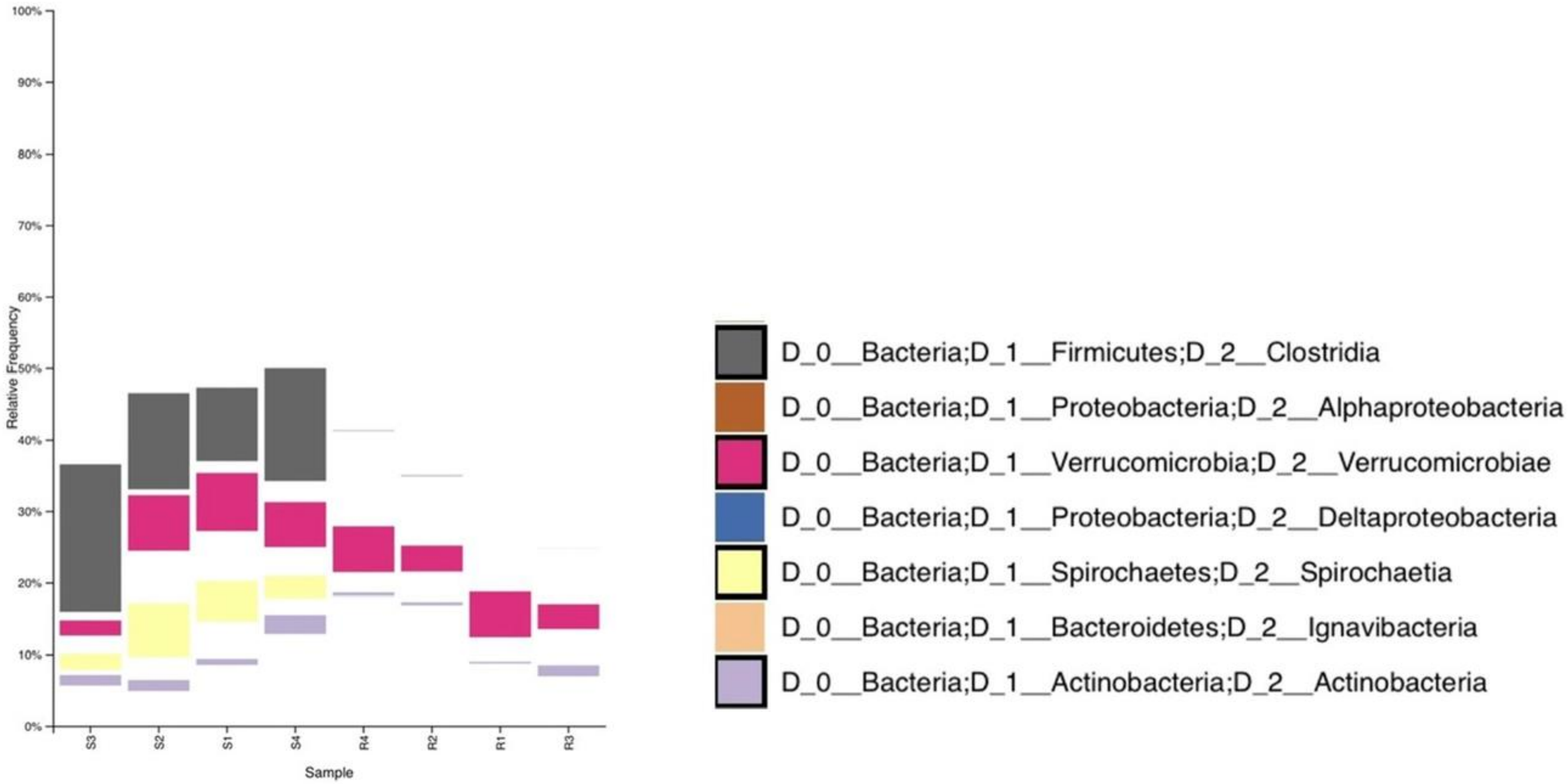

Figure 4.6 Microorganisms found in sediment during bioponics systems with acetic acid added and without acetic acid added (3)

Table 4.4 Microorganisms found in sediment during bioponics systems with acetic acid added and without acetic acid added.

Name	Level	% Abundance		Found at	Role	Reference
		control	acid			
Cellvibrionaceae	family	0.2-0.6%	0-0.1%	Found in water resources	It is a microorganism that helps in degradation.	(X. Zhang et al. 2018)
Pedosphaeraceae	family	2-3%	0-3%	Root surface or root interior	They are bacteria associated with a wide variety of plants and can adapt to the environment.	(W. C. Chen et al. 2021)
Hydrogenophilaceae	family	0.1-2.5%	1-2%	Found in water resources	Help in treating wastewater in the system.	(Al Ali et al. 2020)
Prolixibacteraceae	family	3.2-3.3%	1.2-4.7%	Found in sediment	Degradation of various organic carbons.	(R. Zhang et al. 2019)
Ruminococcaceae	family	1-4%	2-3%	Found in sediment	It is a microorganism that helps in getting rid of diseases that arise from plants.	(Shi et al. 2020)
Firmicutes	phylum	14-17%	11-22%	Found in water resources	It assists in treating wastewater in the system.	(Cazals et al. 2020)

Nitrosomonadaceae	family	0.6-1.6%	0.2-1.4%	Found in water resources	It is a microorganism that helps in the degradation of carbon and nitrites.	(Lüdecke, Nelles, and Dibbert 2020)
Rhodanobacteraceae	family	0.3-2%	0.7-1.5%	Found in sediment	It is a microorganism able to produce biosurfactant In biological treatment	(Kumar, Revathi, and Khanna 2015)
Enterobacteriaceae	family	0.1-0.3%	0%	It can be found everywhere in soil, water, and plants.	It is a pathogenic bacteriam that reduces plant growth.	(Mulaosmanovic et al. 2021)
Gemmatimonadaceae	family	0.6-2%	0.3-0.8%	Found in fertilizers.	It helps in the degradation of potent chitin as a plant disease control agent.	(Hui et al. 2020)
Clostridia	class	13-15%	10-20%	Found in fertilizers.	It is a microorganism that helps in degrading organic matter.	(Ordaz et al. 2019)
Pseudoxanthomonas	genus	1.6-2.6%	0.3-3%	Found in fertilizers.	*Pseudoxanthomonas* can degrade cellulosic and lignocellulosic waste.	(Hu et al. 2021)
Bacteroidales	order	9-16%	16-50%	Found in fertilizers.	It is an indicator of fecal contamination in the system, or it is an indication that the	(Yang et al. 2020)

					chicken manure is in the system.	
Spirochaetia	class	3-7%	2-6%	Found in fertilizers.	It is an indicator of fecal contamination in the system or an indication that the system contains chicken manure. This microorganism can be a microorganism that indicates the disease of the animal.	(M. Zhang et al. 2021)
Verrucomicrobiae	class	6-8%	2-8%	Found in water resources	They are microorganisms indicating external factors that are related to changes in plant plankton communities.	(Y. Chen et al. 2020)
Terrimonas	genus	0.5-3%	0-0.4%	Found in sediment	It is a microorganism used to eliminate unnecessary nutrients.	(M. Zhang et al. 2021)
Actinobacteria	class	1.5-3%	0.8-1.4%	Found in water resources	It assists in treating wastewater in the system.	(Cazals et al. 2020)
Ellin6067	genus	0.6-1.5%	0.2-1.4%	Found in water resources	It assists in treating wastewater in the system.	(Y. Chen et al. 2020)

Herpetosiphon	genus	0-0.3%	0%	Found in water resources	It assists in treating wastewater in the system.	(Livingstone, Morphew, and Cookson 2018)
proteobacteria	phylum	35-40%	16-40%	Found in water resources	It assists in treating wastewater in the system.	(Cazals et al. 2020)

This study found a diversity of microbial communities that contribute as plant growth-promoting bacteria (PGP). Plant growth-promoting bacteria can increase nutrient availability by dissolving it. (For inorganic phosphorus) and mineralization (for organic phosphorus). *Actinobacteria* can dissolve phosphorus and convert phosphorus into a biologically usable form (Yadav & Yadav, 2019). There are microorganisms in sediment during bioponics systems for both acetic acid and non-acetic acid. Both systems had similar percentage exposure. Nevertheless, some microorganisms are more common in acetic acid-enriched systems, such as *Cellvibrionaceae, Pedosphaeraceae, Prolixibacteraceae, Firmicutes, Enterobacteriaceae, Pseudoxanthomonas, Spirochaetia, Verrucomicrobiae, Terrimonas, Actinobacteria, Herpetosiphon,* and *proteobacteria.* It indicates a superlative diversity of microorganisms in systems without acetic acid than one acetic acid-enriched system.

CHAPTER 5

RESEARCH CONCLUSIONS

5.1 Conclusions

The bioponics system without acetic acid added to cos lettuce resulted in optimal plant weight and sufficient phosphorus concentrations for the whole experiment. The system without acetic acid had the highest phosphorus utilization efficiency (3.3%) and the highest plant weight (1194 g). There was no effect of adding acetic acids on the total phosphorus concentrations. Phosphate and COD concentrations and phosphorus transformations occurred in bioponics system by inorganic phosphorus released and available for the growth of plants through the microbial communities in plant roots and sediment. Although the beneficial microbial communities in plant roots are different from the community of sediment microorganisms, multi-genus microorganisms found in this study involved the degradation of organic phosphorus and promoting plant growth. In bioponics system with acetic acid and without acetic acid, the same microorganisms can be found, suggesting that both microbial systems have a wide variety and similar microbial communities. Therefore, growing plants in a bioponic system does not need to add acetic acid to the system in order to adjust the pH to the appropriate level for the plant to grow well. However, plants can be grown by fertilizing and taking care of the system appropriately and growing plants in this system requires care. They keep pests away so they don't interfere with the growth of plants and can be planted in this system for large scale agriculture and trade.

5.2 Research Suggestions

1. The Implementation of very distinct amounts of acetic acid to the bioponics systems to find the best effective relationship for plant growth and nutrient release.
2. Further study of bioponics systems with other different amounts or concentrations of acetic acid to determine the optimal acetic acid content.
3. Bioponics systems should be studied with pathogenic bacteria to be confident that the vegetables in bioponics systems were free from pathogens, safe, and hygienic.
4. Analysis of the economic benefit of different bioponics systems.

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