โครงการ
การเรียนการสอนเพื่อเสริมประสบการณ์

ชื่อโครงการ Bioponic Innovation for Waste-to-Crop: Effects of Phosphorus and COD Concentrations on Microbial Community and Plant Productivity

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ภาควิชา Environmental Science

ปีการศึกษา 2019

คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

SENIOR PROJECT

Project title	Bioponic Innovation for Waste-to-Crop: Effects of Phosphorus and COD Concentrations on Microbial Community and Plant Productivity
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Department	Environmental Science
Academic year	2019

Faculty of Science, Chulalongkorn University

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Bioponic Innovation for Waste-to-Crop: Effects of Phosphorus and COD Concentrations on Microbial Community and Plant Productivity

By

Suchana Amnuaychaichana

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

Faculty of Science, Chulalongkorn University

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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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ภาควิชา	วิทยาศาสตร์สิ่งแวดล้อม	
ปีการศึกษา	2562	

บทคัดย่อ

ระบบไบโอโปนิกส์เป็นการใช้ของเสียอินทรีย์ร่วมกับการปลูกผักแบบระบบไฮโดรโปนิกส์เพื่อเป็นการนำสารอาหารในปุ๋ยอิทรีย์กลับมาใช้ใหม่ทดแทนปุ๋ยอนินทรีย์ที่ใช้ในระบบไฮโดรโปนิกส์ ในระบบไบโอโปนิกส์มีจุลินทรีย์ที่สามารถเปลี่ยนสารอินทรีย์ให้อยู่ในรูปที่พืชสามารถใช้งานได้ ในการศึกษาครั้งนี้เราใช้ Next – Generation Sequencing ในการดูกลุ่มประชากรของแบคทีเรียและศึกษาเกี่ยวกับความสัมพันธ์ระหว่างกลุ่มของแบคทีเรียกับความเข้มข้นของฟอสฟอรัสและซีโอดีโดยการปลูกผักกรีนคอส (*Lettuca sativa*, 18 ต้น/ระบบ) ในระบบไบโอโปนิกส์และใช้ปุ๋ยมูลไก่ (250, 375, และ 500 กรัม) จาการทดลองทั้งหมดพบว่าปริมาณปุ๋ยมูลไก่ที่ 500 กรัมต่อ 18 ต้น ระบบมีค่าประสิทธิภาพในการใช้ฟอสฟอรัส (23.2%) มากที่สุดและได้ผลผลิตที่มีน้ำหนักแห้งมากที่สุด (112 กรัม /18 ต้น) ปริมาณของปุ๋ยมูลไก่ (250 – 500 กรัม) ไม่เกิดความแตกต่างของความเข้มข้นของฟอสฟอรัสทั้งหมด, ความเข้มข้นของซีโอดี และอุณหภูมิในระยะยาว ความสัมพันธ์แบบเส้นตรงของซีโอดีกับฟอสเฟต (r=0.7177) และฟอสฟอรัสทั้งหมด (r=6984) อยู่ในระดับปานกลางนอกจากนี้ในการทดลองยังพบ *Actinobacteria* (ไฟลัมของแบคทีเรีย), *WD2101_soil_group* (แฟมิลี่ของแบคทีเรีย), *Nocardiopsis* และ *Gemmata* ซึ่งเป็นจีนัสหลักที่พบในระบบไบโอโปนิกส์ซึ่งมีความสามารถในการละลายฟอสฟอรัสและพบ *JG30-KF-CM45* ซึ่งเป็นแฟมิลี่ของแบคทีเรียหลักที่พบในน้ำและในตะกอนของปุ๋ยมูลไก่ซึ่งสามารย่อยสลายคาร์บอนอินทรีย์ได้ จากผลการทดลองของการศึกษาครั้งนี้ทำให้สามารถนำไปพัฒนาประสิทธิภาพของระบบไบโอโปนิกส์ที่ใช้ปุ๋ยมูลไก่ในการปลูกผักกรีนคอสได้

คำสำคัญ: ระบบไบโอโปนิกส์, ปุ๋ยชีวภาพ,ประสิทธิภาพของพืชในการนำฟอสฟอรัสไปใช้, การละลายฟอสฟอรัส,ปุ๋ยมูลไก่,กลุ่มจุลินทรีย์

Title **Bioponic Innovation for Waste-to-Crop: Effects of Phosphorus and COD Concentrations on Microbial Community and Plant Productivity**

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Project adviser Dr. Sumeth Wongkiew

Department Environmental Science

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Abstract

Bioponic systems is the combination of the organic waste compost with a closed recirculating hydroponic system to recover nutrients from organics fertilizer instead of hydroponic inorganic solutions. In bioponic systems, microorganisms transform organics nutrients to the bioavailable forms for plant use. This study investigated the microbial communities using Next – Generation Sequencing and examined the linking of microbial abundance to phosphorus and chemical oxygen demand (COD) by planting Romaine lettuce (*Lettuca sativa*, 18 plants/system) with chicken manure compost (250, 375, and 500 grams) in bioponic systems. From all of the experiments, the chicken manure loading rate of 500 g/18 plants (phase 2) had the highest phosphorus use efficiency (23.2%) and the highest plant dry weight (112.8 g/18 plants). The amount of chicken manure loadings (250 – 500 g) did not make different the total phosphorus concentration, the COD concentration and temperature in the long term. COD and phosphate concentrations were positively correlated (r=0.7177) as same as COD and total phosphorus concentration (r=0.6984). Furthermore, this study found the *Actinobacteria* (baterial phylum), *WD2101_soil_group* (bacterial family), *Nocardiopsis*, and *Gemmata* that were the dominant bacterial genera in bioponic system. These bacteria could facilitate phosphorus solubilization. This study also found *JG30-KF-CM45* that was the dominant bacterial family in the water and the compost in chicken manure. It can degrade the organic carbon. The result of this study can improve the performance of chicken manure based bioponic systems for planting lettuce.

Keywords: Bioponics systems, Bio-fertilizer, Phosphorus solubilization, Chicken manure, Microbial community

ACKNOWLEDGEMENTS

First of all, this research would not have been achievable without the kind support and guidance. I take opportunity to express my sincere thanks to all of them.

I deep gratitude to my advisor, Dr. Sumeth Wongkiew, Ph.D., guidance me all the time of research and writing this project. Without his guidance, this project would not have been achievable.

I would like to thank the research committee, Associate Professor Nuta Supakata, Ph.D. and Assistant Professor Pasicha Chaikaew, Ph.D. for the kindness and valuable suggestions.

I would like to thank my laboratory colleagues, Miss Ganisa Prasertsri for our team work and assistance in measurement processes.

I would like to thank to the Department of Environmental Science, Faculty of Science for research facilities and their supports from all staffs.

Last but not the least, I would like to thank my parent and my brother for the all support and encouragement throughout my years of study. I am so grateful to have such an incredible family. Thank you.

Suchana Amnuaychaichana

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CHAPTER 1

INTRODUCTION

1 Significance of the Research

The amount of chemical fertilizer used in agricultural systems has increased to meet the demand of agricultural products. This is based to the fact that the world population has been increased substantially from 7.7 billion in 2019 to 9.7 billion in 2050, according to the United Nation (UN) report. With this growth, the demand of agro-food production will increase and is expected to exceed the food supply. The demand of agro-food production has increased the use of inorganics phosphorus fertilizers from phosphate rock (Horta et al., 2018). Therefore, phosphorus is a major nutrient in agriculture, but non-renewable, which is expected to be limited in the future, suggesting the possible serious concern in the coming decade (European commission,2019).

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 use in agricultural systems can be applied for crop productions using either mineral fertilizers from phosphate rock or organic waste compost (Syers, Johnston, & Curtin, 2008). Organic fertilizer from animal manure was used extensively as organic waste compost to recovery nutrients such as nitrogen and phosphorus in agriculture (Mullins, 2009). Chicken manure has high concentrate of phosphorus, nitrogen and soil organic carbon that are essential for plants growth in organic farming. (Ravindran, Mupambwa, Silwana, & Mnkeni, 2017).

The reactive phosphorus loss from intensive fertilized cropland has affected the worldwide agricultural systems and the environments (da Silva Cerozi & Fitzsimmons, 2016a) such as the accumulation of phosphorus in soil (Jing, Christensen, Sørensen, Christensen, & Rubæk, 2019) and eutrophication in surface water (Mullins, 2009). In addition, mining phosphorus rock, processing phosphate fertilizer and long-term use of phosphorus fertilizer in cropland can lead to the accumulation of trace elements such as As, Cd and Pb in soil (Jiao, Chen, Chang, & Page, 2012).

Combination of the organic waste compost with a closed recirculating hydroponic system, called bioponics, is an innovative food production system that produces organic vegetables to meet the demand of agro – food productions and decrease the impacts of phosphate fertilizer on the environment. Bioponics is similar to hydroponics, but bioponics uses organics fertilizer instead of hydroponic inorganic solutions. Bioponics also recovers nutrients from organic fertilizers that are generally made from agricultural residues. Unlike hydroponics, bioponic systems are abundant of microbial communities that degrade organics nutrient to available nutrients for plants (Saijai, Ando, Inukai, Shinohara, & Ogawa, 2016). Bioponic systems consist of a biofilter, where the microorganisms the in biofilter degrade the organic waste compost to the available nutrients for assimilation by plants.

There are several microbial genera responsible in phosphorus transformations in biological processes, which can occur as similarly as bioponics. There are several enzymes produced from microorganisms to utilize 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 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 decrease removal efficiencies of chemical oxygen demand (COD) by heterotrophic bacteria (Zheng et al., 2019). Heterotrophic bacteria are the group of bacteria that degrades organic carbon and decreases COD concentration. Moreover, the different environment conditions such as oxygen concentration, pH and nutrient availability can shift the microbial communities. (Wongkiew, Park, Chandran, & Khanal, 2018). Thus, to improve phosphorus use efficiency and plant productivity in bioponics, understanding of COD concentrations, pH, and microbial community are necessary that can be key for reviewing processes in bioponic systems.

Recently, there are studies about using compost teas (compost mixed with animal manure applied with beneficial microorganisms) in hydroponics and determining the impact of manure-based extracts on plant yield in hydroponic (Tikasz, MacPherson, Adamchuk, & Lefsrud, 2019). However, there are still few studies that use the biofilter in hydroponic system (bioponics) that apply animal manure, particularly phosphorus-rich chicken manure, and examine the link between microbial communities and phosphorus transformation to improve phosphorus use efficiency and increase plant productivity. Such study can be guidelines for farmers, who are interested in bioponic system or organic-waste recovery, to perform organic waste recycling from waste-to-food, suggesting a new innovative approach in the bioeconomy, circular economy, and green economy (BCG) model.

The overarching goal of this study is to examine the link between microbial communities, phosphorus and COD concentrations. The specific objectives are to 1.) Examine the effects of chicken manure loading rate and waste-to-plant ratio on phosphorus and COD concentrations in bioponic systems and phosphorus use efficiency in plants. 2.) Examine the microbial community in bioponics and link the microbial abundances to phosphorus and COD availabilities. This study aims to improve the performance of bioponic systems, which reduce the amount of mineral fertilizer used and reduce the effects of the reactive phosphorus loss throughout the environment that is a cause of eutrophication in surface water and accumulation of phosphorus in soil.

1.2 Research Objectives

1.) Examine the effects of chicken manure loading rate and waste-to-plant ratio on phosphorus and COD concentrations in bioponic systems and phosphorus use efficiency in plants.
2.) Examine the microbial community in bioponics and link the microbial abundances to phosphorus and COD availabilities.

1.3 Expected Outcomes

1) Knowing the effect of microbial abundances to improving phosphorus and COD availabilities
2) The appropriate waste-to-plant ratio could be used in bioponic systems
3) Improving the process to be highly efficient bioponic systems

CHAPTER 2

LITERATURE REVIEWS

2.1 Substrates for plant growth

Substrates for plant growth can provide nutrient for plants, which can be categorized into three types as follow (Agricultural Economics, n.d.).

2.1.1 Chemical fertilizer

Chemical fertilizer is inorganic compound that be applied to increasing soil nutrient (e.g., hydroponic nutrient solution, urea fertilizer).

2.1.2 Organic substrates

Organic fertilizer is a natural product of organic compounds. It can be produced from animal manures and dead leaves or plants (e.g., raw animal manure, leaves, straws, wood, food waste, agriculture residues). This type of fertilizers needs microbial degradations to prevent heat damage on plants before effectively used for crop production.

2.1.3 Bio-organic fertilizer

Bio-organic fertilizer is an organic fertilizer type that is already produced from microbial processes. Bio-organic fertilizer process can synthesize plant nutrients or transform organic nutrient to bio-available nutrient forms (e.g., composts made from organic waste). Bio-organic fertilizer is organic fertilizer produced at high temperature under aerobic microbial degradation. The high temperature can deactivate harmful microorganisms that cause plant, animal and human diseases. Bio-organic fertilizer can be then added with plant growth promoters to improving organic fertilizer. Plant growth promoter is a group of bacteria that can enhance available nutrients. In bioponics, types 2 and 3 fertilizers can be used as the nutrient sources for the plants. Microorganisms in bioponic biofilters can allow the microbial processes to degrade organic compounds and possibly remove diseases from the disease-contaminated fertilizers although high temperature is not build-up.

Table 2.1 Example of Animal manure fertilizer nutrient contents

Animal manure fertilizer	Nutrient(%w/w)		
	Nitrogen	Phosphorus	Potassium
Cow manure	1.10	0.40	1.60
Pig manure	1.30	2.40	1.00
Chicken Manure	2.42	6.29	2.11
Bat manure	1.54	14.28	0.60

(Available from: http://www3.oae.go.th/rdpcc/images/filesdownload/km/Knowledge/productions/13.pdf).

2.2 Biofilters and grow beds

Biofilters are the technology to treat pollutants (e.g., soluble organic matters, suspended solids, nutrients) using microbial degradation. In bioponics, Biofilters are used for microbial attachment, allowing high surface area for biochemical reactions. Microbial communities can degrade organic substrates from organic fertilizer, releasing available nutrients for plants. Sufficient nutrients available for plants can increase nutrient use efficiency and enhance plant growth and plant yield (Saijai et al., 2016).

There are six types of bioponics categorized based on types of grow bed (“Basic Hydroponic Systems and How They Work”,2019).

2.2.1 Wick system

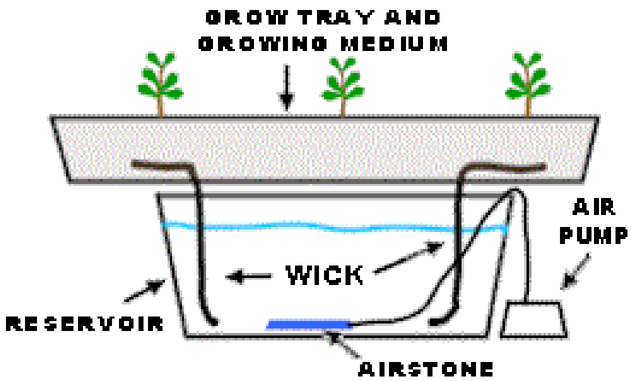

Figure 2.1 Wick system (Available from: https://www.simplyhydro.com/system/)

2.2.2 The water culture system

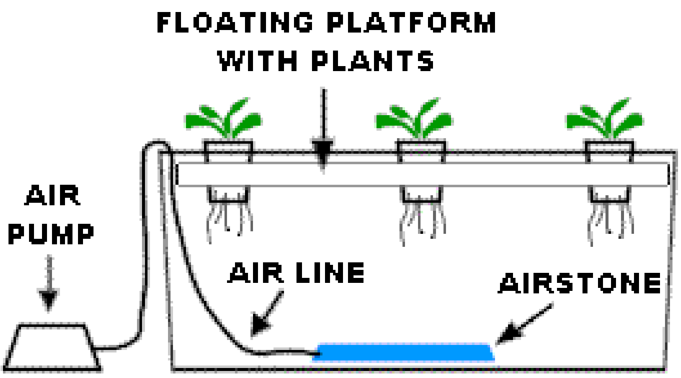

Figure 2.2 The water culture system (Available from: https://www.simplyhydro.com/system/)

2.2.3 Flood and drain system

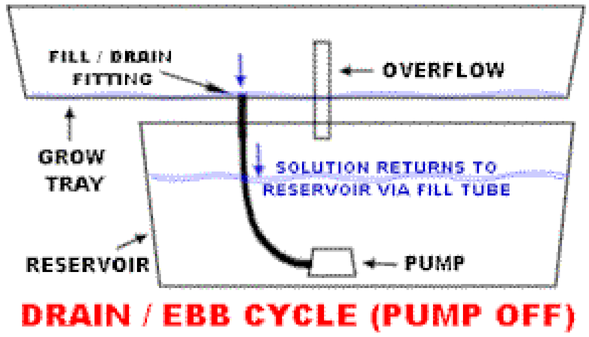

Figure 3. Flood and drain system (Available from: https://www.simplyhydro.com/system/)

2.2.4 Drip systems

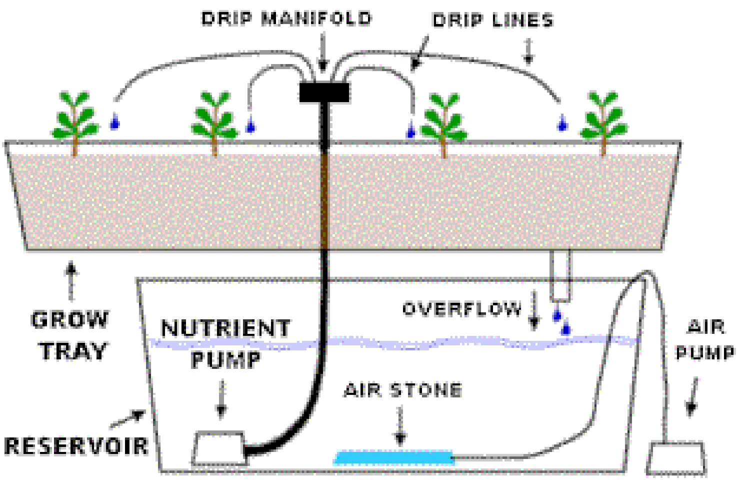

Figure 4. Drip systems (Available from: https://www.simplyhydro.com/system/)

2.2.5 Nutrient Film Technique (NFT)

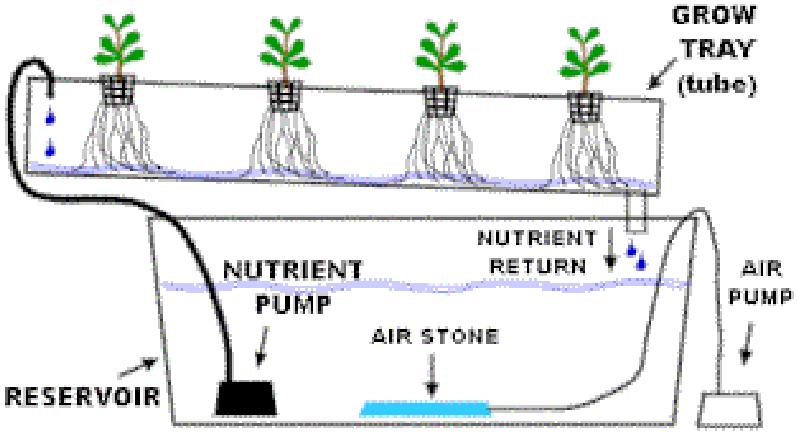

Figure 5. Nutrient Film Technique (NFT) (Available from: https://www.simplyhydro.com/system/)

2.2.6 Aeroponic system

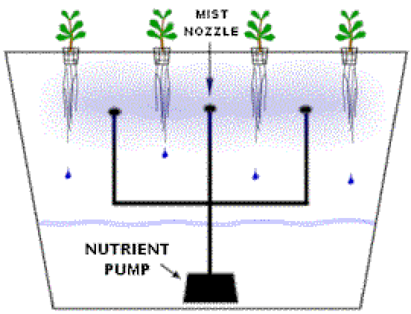

Figure 6. Aeroponic system (Available from: https://www.simplyhydro.com/system/)

2.3 Microorganisms

Bioponics are the nutrient recovery technology that combines composting with hydroponics. Although microbial community in bioponic systems are still not yet studied, it may have some similarity to microbial community in aquaponic systems, which have microorganisms to transform organic nutrient to available form. Bioponics are similar to aquaponics as both systems release ammonia nitrogen and organic carbon from animal waste. Aquaponics utilizes fish waste while bioponics utilizes other animal wastes. In aquaponics systems, a study showed that microbial communities from biofilter and plant roots were investigated by Next-Generation Amplicon Sequencing and quantitative polymerase chain reaction analyses (Wongkiew et al., 2018). This study showed the differences of microbial communities in biofilters and plant roots.

In plant root, plant growth promoters rhizobacteria (PGPR) are root-associated bacteria that can increase nutrient availability (da Silva Cerozi & Fitzsimmons, 2016b). PGPR includes three type such as free-living bacteria (around of the root), bacteria colonize the root surface, and endophytic bacteria that live within plant root. There are several microbial communities that are PGPR, such as nitrifiers and phosphorus solubilizing bacteria (Ruzzi & Aroca, 2015). Nitrifiers can transform ammonia nitrogen to nitrate nitrogen (Wongkiew, Hu, Chandran, Lee, & Khanal, 2017), while phosphorus solubilizing bacteria can solubilize precipitated inorganic phosphorus (Richardson & Simpson, 2011) to soluble phosphate, which is the bioavailable phosphorus form for plant use (Alori et al., 2017). The dominant species in rhizosphere are *Bacillus*, *Pseudomonas striata*, and *Enterobacter* that can mineralize organic phosphorus and solubilize precipitated phosphorus (da Silva Cerozi & Fitzsimmons, 2016b). In biofilter, *Cetobacterium* spp. (anaerobe) and *Comamonadaceae* (aerobe) are the dominant species. *Cetobacterium* spp. (anaerobe) are denitrifiers that can reduce nitrate, leading to nitrogen loss but it can degrade organic compounds in sediment and decease by-product value as an organic fertilizer. Ammonia-oxidizing bacteria and nitrite-oxidizing bacteria can be found both in biofilter and rhizosphere, suggesting that nitrogen transformation and improving nitrogen use efficiency (Wongkiew et al., 2018). These types of microorganisms may associate with phosphate-releasing and organics-degrading bacteria, which will be studied in this study.

2.4 Microbial degradation

Microbial degradation is the breakdown of organic compounds by microorganism such as bacteria and fungi. Mineralization is the complete biodegradation to breakdown the complex molecule to element form. Partial biodegradation is the term of biodegradation that breakdown of organic compound into less complex compound. The production of microbial degradation is oxygen consumption, carbon dioxide formation, and substrate loss ("Chapter 7 - Environmental Issues," 2003). Organic compounds are the carbon source for Heterotrophic. Chicken manure has high concentrate of cellulose (Schnitzer, Monreal, Facey, & Fransham, 2007). Microorganism can degrade cellulose to glucose that heterotroph can use to cell respiration. The microbial degradation mechanism of cellulose uses cellulases enzyme. At least three types of cellulases that use to degrade cellulose consist of Endoglucanase, Exoglucanase and β – glucosidases. First, Endoglucanase produce the random cuts in the cellulose molecule to cello-oligosaccharides. Then Exoglucanase breakdown the end of the cello-oligosaccharide chains to glucose, cellobiose and/or cello-oligosaccharide. At the end, β – glucosidases hydrolyze the soluble cellodextrin and cellobiose to glucose (Lakhundi, 2015).

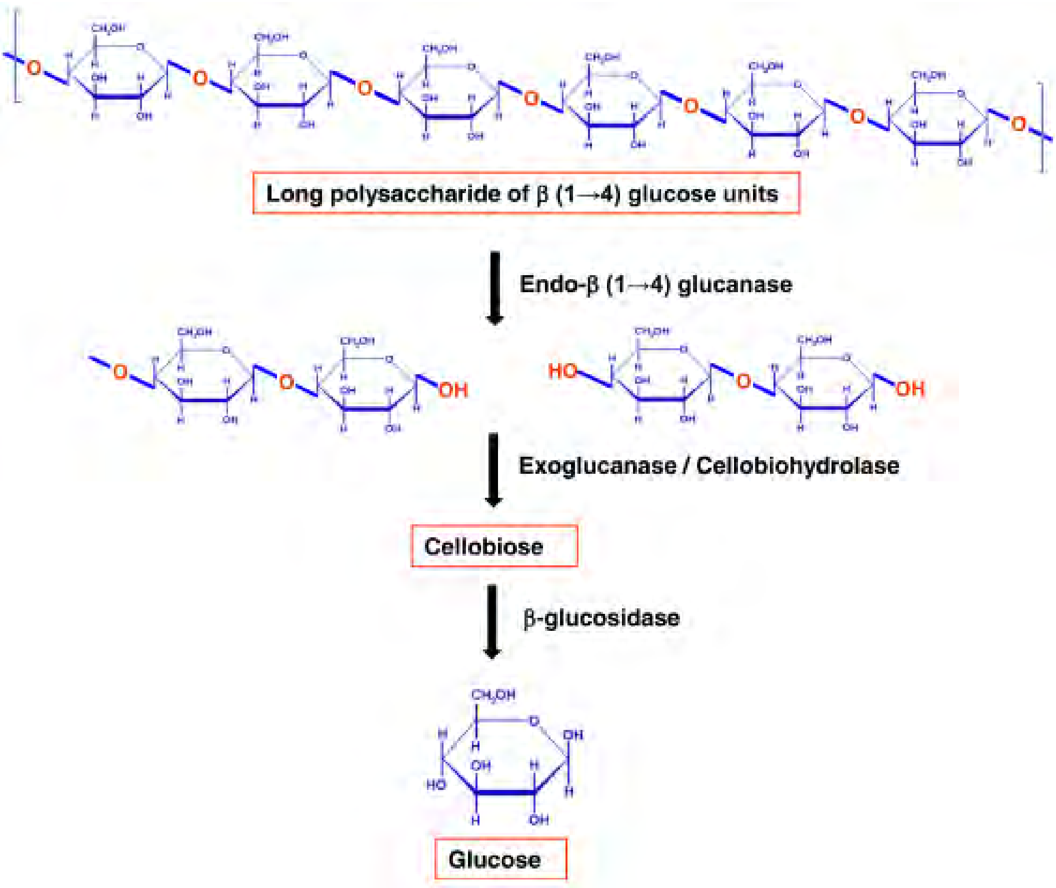

Figure 2.7 Classes of enzymes involved in cellulose breakdown. (Lakhundi, 2015)

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 (Figure 3.1). A bioponic system consisted of one recirculating tank for aeration and recirculation water (~18 liters), one up-flow biofilter (~18 liters), and three-channel grow bed (18 plants per one bioponic system) (Figure 8). Dry chicken manure were applied in the biofilter and 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). Romaine lettuce (*Lactuca sativa*), aka Cesar salad, were used as tested vegetables in this study. Romaine lettuce is one of the most popular fresh organic vegetables (Demir, 2019). Biochemical filter sponges were used to increase surface area for microbial attachment in the up-flow biofilter. The bioponic systems were operated in triplicate (n=3).

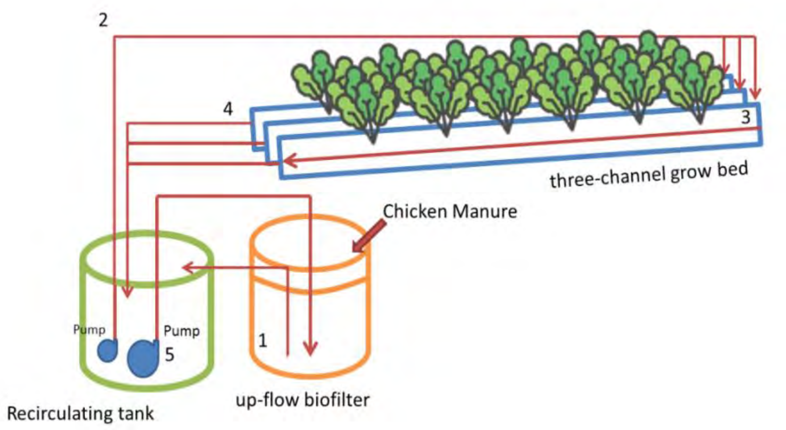

Figure 3.1. Schematic diagram of a bioponic system in this study, consisting of biofilter (1), nutrient feeding line with pump (2), NFT grow bed (3), water outlet back to recirculating tank (4), and biofilter water-feeding pump

3.2 Approaches

Objective 1: Examine the effects of chicken manure loading rate and waste-to-plant ratio on phosphorus and COD concentrations in bioponic systems and phosphorus use efficiency in plants.

Rational: Chicken manure is the major source of phosphorus in bioponic systems. Phosphorus is the primary nutrient for plants. COD is the parameter representing the amount of organic compound, which is the major source of carbon. Microorganisms breakdown of the organic compounds and use the products in cell respiration, producing nutrients for plants. The experiments for optimizing suitable weight of chicken manure (organic loading rate) can improve the phosphorus use efficiency and plant productivity of bioponic systems. To make the organic fertilizer guideline for bioponic users, appropriate waste-to-plant ratio can be calculated from an optimized organic loading rate. This is a practical value for scaling up the bioponic systems.

Hypothesis: Phosphorus is the primary nutrient for plant, and microorganism need organic compound to produce nutrients for plant. If chicken manure loading is increased in bioponics, then phosphorus and organic compound (representing as COD) will increase and improve nutrient use efficiency in bioponic system. However, the excessive increase of chicken manure loading could be cause of phytotoxic for the plant (Hawkesford et al., 2012).

Task: Romaine lettuce (*Lactuca sativa*) were planted three rounds with different chicken manure loading (250 g (phase 1), 500 g (phase 2), and 375 g (phase 3, confirmative test)). Each round took 5 weeks for plant growth in bioponics. Seed germinations were taken about 3 weeks before transplantations to grow bed. Water in bioponics were sampled and were monitored weekly for total phosphorus (TP), 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 will be monitored using a pH meter. Bioponic systems were operated in triplicate. At the end of each experiment, all plants were harvested and weighed for wet weight and moisture content. Plants and organic fertilizer samples were sent to Food research and testing laboratory (FTRL), Chulalongkorn University for analyses of nitrogen and phosphorus contents. Once the optimal range of organic loading rates were found, the optimal organic loading was retested in the bioponics to ensure that was an acceptable condition (confirmative test, phase 3). In phase 3, chicken manure of 375 g was used because it was in the middle of effective range of chicken manure loading for growing lettuce (250-500 g) Moreover, in phase 3, operating time of 4 weeks was used as it is the minimal time for growing lettuce to sell in market. The results were statistically tested using one-way analysis of variance (ANOVA) followed by Tukey's test (between multiple groups, $\alpha = 0.05$) to compared the amount of DO, temperature, phosphate concentration, total phosphorus concentration, COD concentration, plant dry weight and phosphorus use efficiency in three phases of the experiment. The correlations of COD and phosphate was examined by using Pearson correlation coefficient as same as the correlation of COD and total phosphorus.

Objective 2: Examine the microbial community in bioponics and link the microbial abundances to phosphorus and COD availabilities.

Rational: Microorganisms are the most important in bioponic biofilters to degrade organic phosphorus in chicken manure. The microbial degradation will allow the generation of bioavailable phosphorus for plants. Organic compounds (representing as COD) are also important for heterotrophic bacteria because organic compounds are the source of carbon that is used in metabolism pathway. Heterotrophic bacteria degrade organic carbon and organic phosphorus. Dissolve organic carbon utilizers play a significant role to stimulating and increasing the phosphate concentrations.

Hypothesis: There could be some dominant microorganisms related to the phosphorus transformation and COD concentrations in bioponic systems. For example, *Enterobacter* spp. could be dominant species in bioponic systems because they could be important for phosphorus solubilization, generating phosphate that can be used by plant. Microorganisms degrade and utilizes organic carbon from chicken manure. Organic compounds (representing as COD) could be utilized by heterotrophic bacteria in bioponic systems. The degradations of organic phosphorus, organic compounds and the utilization of phosphorus could be correlated with some specific microbial groups in different bioponic components such as plant roots, recirculating water, and biologically degraded chicken manure in biofilters. This part will help us to find dominant species that could be enriched in bioponics for the highest phosphorus generation and maintain good water quality for plants and its productivity.

Task: At the end of each experiment, water, biologically degraded chicken manure, and plant root samples were 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. All of the samples were sent to Omics Center, Chulalongkorn University. Raw sequence read were analyzed for operational taxonomy units (OTUs) at genus level and microbial diversity using QUIIME software by Omics Center. Microbial abundances were calculated based on sequence reads.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Bioponic system performance

The bioponic systems used chicken manure compost in closed recirculation hydroponic systems and had a good performance. The plants grew effectively and had suitable weight (1000 – 2100 g/18 plants) phosphorus content (0.83% w/w) in the plant biomass. The water in bioponic system had high dissolve oxygen (DO) concentration (6.3-7.1 mg/L), slightly basic (8.04-8.68) and warm temperature (30.7- 32.1 °C). The phosphorus concentration in the water and the organic carbon presented by COD concentrations were sufficient for the plant growth (Table 4.1).

Table 4.1. Bioponics performances and water quality in bioponic systems

Phase	DO (mg/L)	pH	Temp. (°C)	Phosphate (mg/L)	TP (mg/L)	COD (mg/L)	Plant dry weight (g)	PUE
Phase1 (250 g)	6.7 ± 0.5^{ab}	8.04 - 8.68	30.7 ± 1.0^{a}	33.32 ± 8.15^{a}	46.50 ± 16.71^{a}	320.08 ± 141.51^{a}	57.3 ± 8.2^{a}	17.0 ± 2.4^{a}
Phase2 (500 g)	6.3 ± 0.8^{a}	8.21 - 8.62	31.2 ± 0.8^{a}	57.35 ± 16.93^{b}	70.71 ± 26.75^{a}	380.78 ± 79.00^{a}	112.8 ± 9.6^{b}	23.2 ± 2.0^{b}
Phase3 (375 g)	7.1 ± 0.9^{b}	8.36 - 8.62	32.1 ± 0.9^{a}	39.94 ± 11.12^{ab}	46.18 ± 15.89^{a}	224.92 ± 113.81^{a}	53.0 ± 7.9^{a}	12.2 ± 1.8^{a}

Values are the mean ± standard deviation (n=3) for plant dry weight and PUE. The superscripts a and b represent statistical difference ($p < 0.05$). Comparisons were made within each parameter. Days of operation: phases 1 and 2 (5 weeks) and phase 3 (3 weeks). Phosphorus use effeciency (PUE) were measure by calculating (PUE = phosphorus uptake by plant/ ∆Quantity of phosphorus applied ×100)

Table 4.1 showed pH levels and temperatures from three phases of bioponic systems were not significantly ($p \geq 0.05$) diferrent from each other (phases 1, 2 and 3). This can be suggested that the plants in bioponic systems were planted at a similar environment and the amount of chicken manure loading (250 g – 500 g) did make different on the temperature in the long term.

For phosphate concentration, in phase 1 (250 g dry mannure), the phosphate concentrations was significantly ($p < 0.05$) lower than phosphate concentrations in phase 2 (500 g) because the amount of chicken manure loading in phase 1 was lower than the amount of chicken manure loading in phase 2. Thus, the phosphorus concentrations dissolved in the water in phase 2 was higher than phase 1. The organic phosphorus in water solubilized and became the bioavailable phosphorus form by microorganisms in bioponic system. The organic phosphorus was transformed to phosphate, which is the bioavailable phosphorus (Richardson & Simpson, 2011). However, the phosphate concentrations in phase 3 (375 g) were not significantly diferrent ($p \geq 0.05$) from the phosphate concentrations in phase 1 (250 g) and phase 2 (500 g) because the amount of chicken manure loading in phase 2 (375 g) was in the middle point between phase 1 and phase 3. The phosphate concentrations (in phase 2) were transformed from organic phosphorus by microorganisms, thus showing similar phosphate concentrations to phase 1 and phase 3. Other study used the lettuce planting in aquaponic system that had similar phosphorus concentrations as this experiment. The phosphorus content in plants in that study was 0.91% w/w (Cerozi & Fitzsimmons, 2017). By comparison, the average of phosphorus content in this study was 0.83% w/w. This suggested that bioponic system had the good performance compared with other literatures.

By comparison, average value of total phosphorus (TP) concentrations in phase 1 was not significantly ($p \geq 0.05$) different from TP concentrations in phase 2. In addition, the TP concentrations in phase 3 were not significantly ($p \geq 0.05$) different from the TP concentrations in phase 1 (250 g) and phase 2 (500 g). It can imply that the amount of chicken manure loading (250 to 500 g) did not make different on the phosphate concentration in the long term. In addition, the COD concentrations in the three phases of the experiments were not significantly ($p \geq 0.05$) different from each other, similarly to the phosphorus concentrations. Thus, the amount of chicken manure loading did not make different on COD concentrations during the plating peroid. Other study evaluated the effect of pH on phosphorus availability in aquaponic system. It was found that pH had effect on the transformations of phosphorus and its bioavailable forms. the phosphorus that have the highest tranformations rate have pH = 8.5(da Silva Cerozi & Fitzsimmons, 2016a). Therefore, bioponic systems have a suitable pH to transformed the phosphorus to the bioavailable form.

The plant dry weight in phase 2 was significantly ($p < 0.05$) different from the plant dry weight in phase 1 and phase 3 as the amount of chicken manure loading in phase 2 (500 g) was higher than the amount of chicken manure in phase 1 (250 g) and phase 3 (375 g). Therefore, the phospohorus concentration in phase 2 was higher than the phosphorus concentrations in phases 1 and phase 3. This suggested that that using chicken manure loading below 250 grams might lead to nutrition deficiency that could affect plant growth. Therefore, the plant dry weight in phase 1 was significantly ($p < 0.05$) lower than phase 2. However, the plant dry weight in phase 1 was not significantly ($p \geq 0.05$) different from phase 3 as the plants in phase 3 were harvested only 4 weeks after transplaning. The plants in phase 3 had lower time to grow and increase their weight. However, within 4 weeks, plants can yield a sellable biomass with a good size for consumption. Normally, The plant (lettuce) have rapid growth rate and the plant dry weight reached exponential phase after 3 weeks after transplaning (Cerozi & Fitzsimmons, 2017). Therefore, days of planting and the amount of chicken manure (organic loading rate) are important 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 cell membrane. Thus, phosphorus are very important for plant growth (Hawkesford et al., 2012).

The phosphorus use efficiency (PUE) in phase 2 (500 g) was significantly ($p < 0.05$) different from the PUE in phase 1 (250 g) and phase 3 (375 g) as the plant dry weight in phase 2 was higher than phase 1 and phase 3. The phosphorus concentrations that the plants utillized in phase 3 were lower than phase 2 as the plants in phase 3 had lower time to growth. Therefore, the phosphorus concentration in phase 2 that the plant utillized was higher than phase 1 and phase 3. In contrast, the PUE in phase 1 was not significantly ($p \geq 0.05$) different from the PUE in phase 3 because the the plant dry weight in phase 1 was not significantly ($p \geq 0.05$) different from phase 3. Thus, the phosphorus that the plant utillized in phase 1 was similar as phase 3. Furthermore, the plant dry weight in phase 1 (less nutrient loading) was similar as phase 3 (less days of planting). By comparison, other study use the *Bacillus* sp. to increase the performance of 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 this experiment as the phosphorus concentration in chicken manure was higher than fish feed, and the chicken menure compost still contained amount of phosphorus in the bulk sediment.

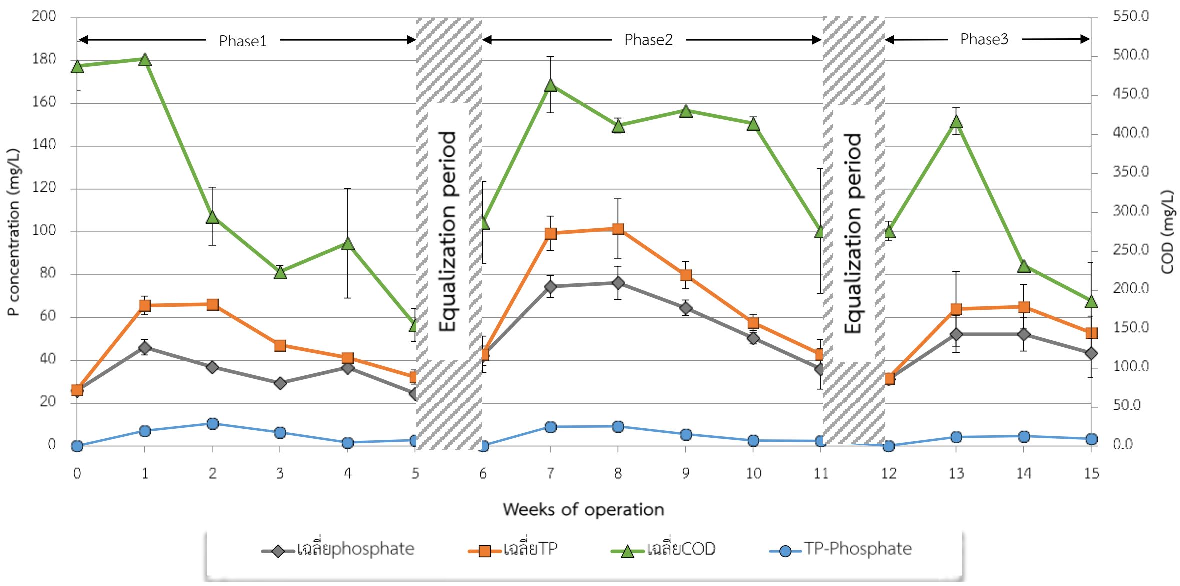

Fig. 4.1 the variation of phosphate, phosphorus, COD and diference of phosphorus and phosphate with different chicken manure (250 g in phase 1, 500 g in phase 2 and 375 g in phase 3) in bioponic systems

4.2 Phosphorus transformations

4.2.1 Total phosphorus and phosphate transformation

This line graph (Fig. 4.1) illustrates the concentrations of total phosphorus (TP), phosphate and COD in bioponic systems in phases 1, 2 and 3 with different chicken manure loading rates (250 g in phase 1, 500 g in phase 2 and 375 g in phase 3) in 5 weeks after transplaning.

In phase 1, the TP concentration showed the same pattern as TP in phases 2 and 3, but the phosphate concentrations were different from the others. During the first week after transplaning, the amount of chicken manure dissolved in water, and phosphate uptake by plant were low becasue phosphorus generation by the mannure required 2-3 weeks to provide maximum concentrations. Therefore, the TP concentration increased dramatically from 26.25 to 65.28 mg/L, and the phosphate concentration also increased from 26.01 to 46.13 mg/L. After that, the TP concentration increased slightly to 66.24 mg/L in the second weeks and droped to 32.27 mg/L at the end of operation (week 5). By comparison, the concentrations of phosphate dropped sharply to 29.49 mg/L in the third weeks as the plants assimilated the phosphate with a higher rate than the phosphate generation rate. However, the concentration of phosphate increase to 36.72 mg/L then drop to the lowest point at 24.62 mg/L in the end (week 5). Comparing to this study, other study used the lettuce and planting in aquaponic system. The phosphate concentration increased dramatically in the first week. Then, the phosphate concentration reach to the peak point in week 3 and decrease shraply after third week (Cerozi & Fitzsimmons, 2017).

Nevertheless, during the first week of phase 2 after transplaning, the TP concentration increased dramatically from 42.91 mg/L to 99.37 mg/L due to a higher phosphorus released from the mannure. The concentration of phosphate also increased sharply from 42.22 to 74.53 mg/L in the first week. After that, the TP and phosphate concentrations rose gradually to the highest point of 101.59 and 76.27 mg/L, respectively, from the second weeks after transplaning as the phosphate was taken up by plants, which was nearly equivalent to the phosphate generation rate. However, in the third weeks after transplaning, the plants had rapid growth rate because the plants reached an exponential growth, which can be noticed by fully developed leave and root systems (Cerozi & Fitzsimmons, 2017). Thus, the concentration of TP and phosphate dropped sharply to 42.86 and 36.07 mg/L, respectively at the end of the phase 2. Other studies also found that once plants reach an exponential growth. A maximum nutrient uptake rate can be identfied from the decrease in phosphate accumulation rate (decrease in phosphae concentration in Fig. 4.1) (Cerozi & Fitzsimmons, 2017).

By comparison, the TP and phosphate concentrations in phase 3 had same pattern as phase 2, but the maximum phosphate concentration was lower than phase 2. The TP concentrations increased dramatically in the first week from 31.75 to 64.02 mg/L. The concentration of phosphate also increased from 31.23 to 52.25 mg/L as the phosphate utilization rate in plants was lower than phosphate genration rate. After that, the TP concentrations rose slightly from 64.02 to 65.08 mg/L, and the phosphate concentration was in steady-state (constant phosphorus concentration) in the second weeks because the phosphate was taken up by plants with the same rate as the phosphorus generation rate. However, during the last weeks, the TP and phosphate concentrations decreased dramatically to the lowest point at 43.45 and 52.91 mg/L, respectively, as the plant have rapid growth after the third weeks, which also occurred in phases 1 and 2.

4.2.2 Characteristics of COD and its correlations to phosphorus concentrations

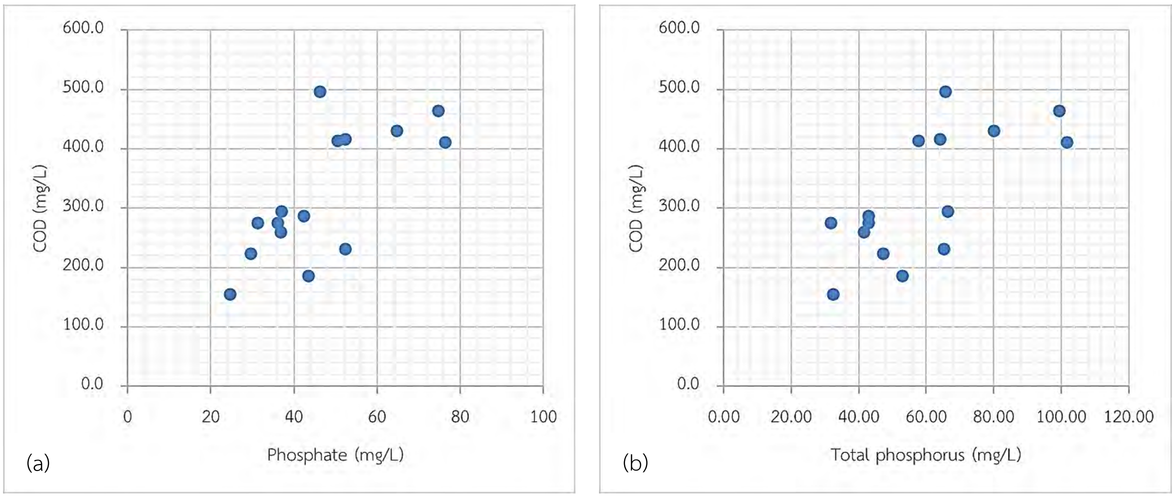

Fig. 4.2 The Correlations of (a) Phosphate and COD and (b) Total phosphorus and COD in bioponic system

Carbon source can affect the production of organic acid (inorganic phosphorus solubilization) and enzyme (organic phophorus mineralization) that can affect the phosphorus solubilization and mineralization by microorganisms (Saif, Khan, Zaidi, & Ahmad, 2014). Chemical oxygen demand (COD) can represent the amount of cabon source in bioponic system (Table 4.1 and Fig. 4.2). In the experiment, The COD concentrations and the phosphate concentrations had the medium positive correlation (r = 0.7177). The COD concentrations and the TP concentrations also had the medium positive correlation (r = 0.6984).

For example, during the first week in phase 1 (250 g) after transplaning, the organic phosphorus in chicken manure solubilized and dissolved in water. Therefore, the COD concentrations increased slightly and reached to a peak from 488.1 to 497.3 mg/L. After that, the COD concentrations decreased dramatically to the lowest point of the phase at 155.6 mg/L in week 5 after transplaning.

However, the COD concentrations in the first week of phase 2 (500 g) increased sharply from 287.3 to 464.1 mg/L after transplaning due to a higher amount of chicken mannure added (Fig 4.1). After that, the COD concentrations decreased slightly to 411.6 mg/L in the second week after transplaning. Then, the COD concentrations were stable with 414.4 mg/L in week 4. However, in the last week after transplaning (phase 2), the concentration of COD decreased dramatically to 276.3 mg/L (Fig 4.1).

By comparison, during the first week after transplaning in phase 3 (375 g), the COD concentration also increased dramatically from 276.3 to 417.2 mg/L. After that, the COD concentration decreased sharply to 95.9 mg/L at the end of the phase (week 5).

Overall, The COD concentrations (phase 1-3) increased in the first week after transplaning. After that, the concentrations decreased dramatically from the second week to the end of the phase (week 5). However, The COD concentrations in three phases were not significantly ($p \geq 0.05$) diferrent. This could be due to that the amount of chicken manure loading (250 g – 500 g) was not in a high amount that could cause an effect on the COD concentration.

4.3 Bacterial community in bioponic systems

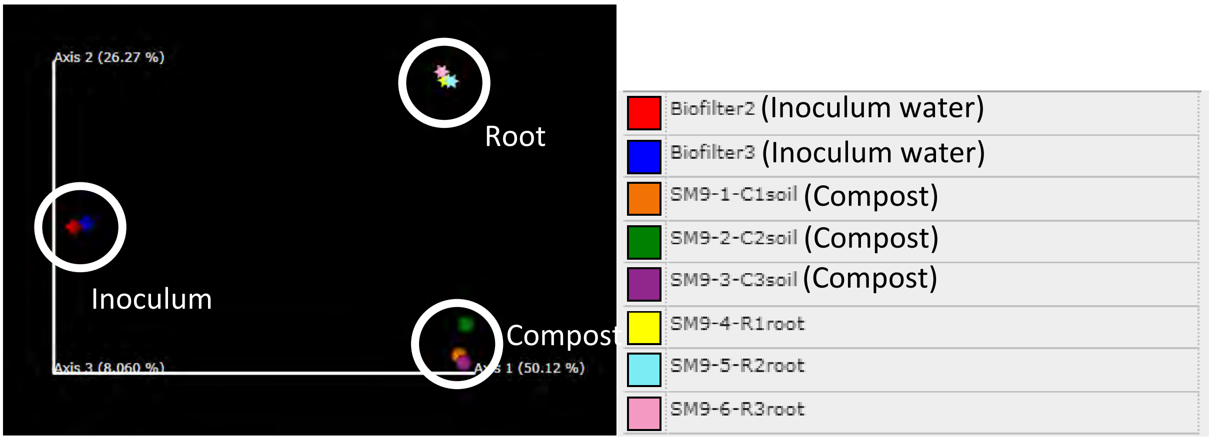

Fig. 4.3 Clusters of Microbial comunities in the water from bioponics system before the planting (Biofilter2 and biofilter3), the root of the lettuce (SM9-4-R1root, SM9-5-R2root and SM9-6-3root) and the compost from the chicken manure after havesting (SM9-1-C1soil, SM9-2-C2soil and SM9-3-C3soil)

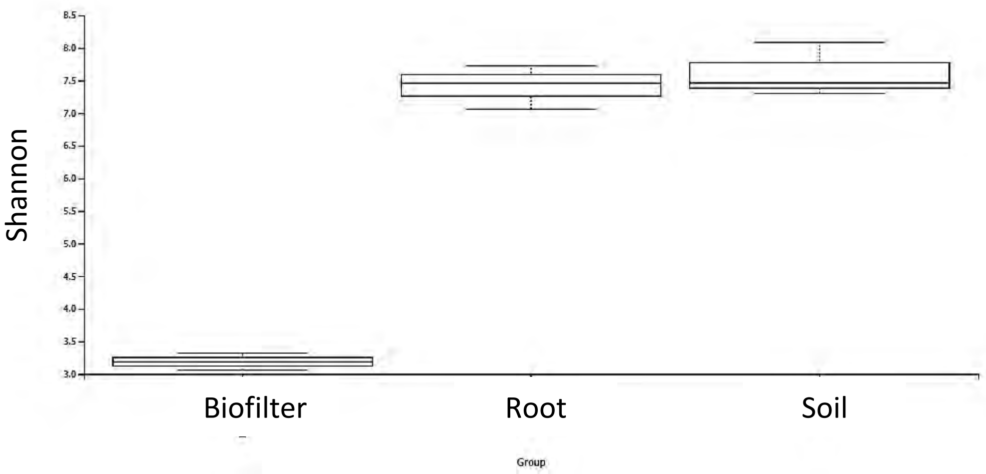

Fig. 4.4 Alpha biodiversity of microbial communities by Shannon - Wieners diversity index (H') in the water from bioponics system before the planting (Biofilter), the root of the lettuce (Root) and the compost from the chicken manure after havesting (Soil).

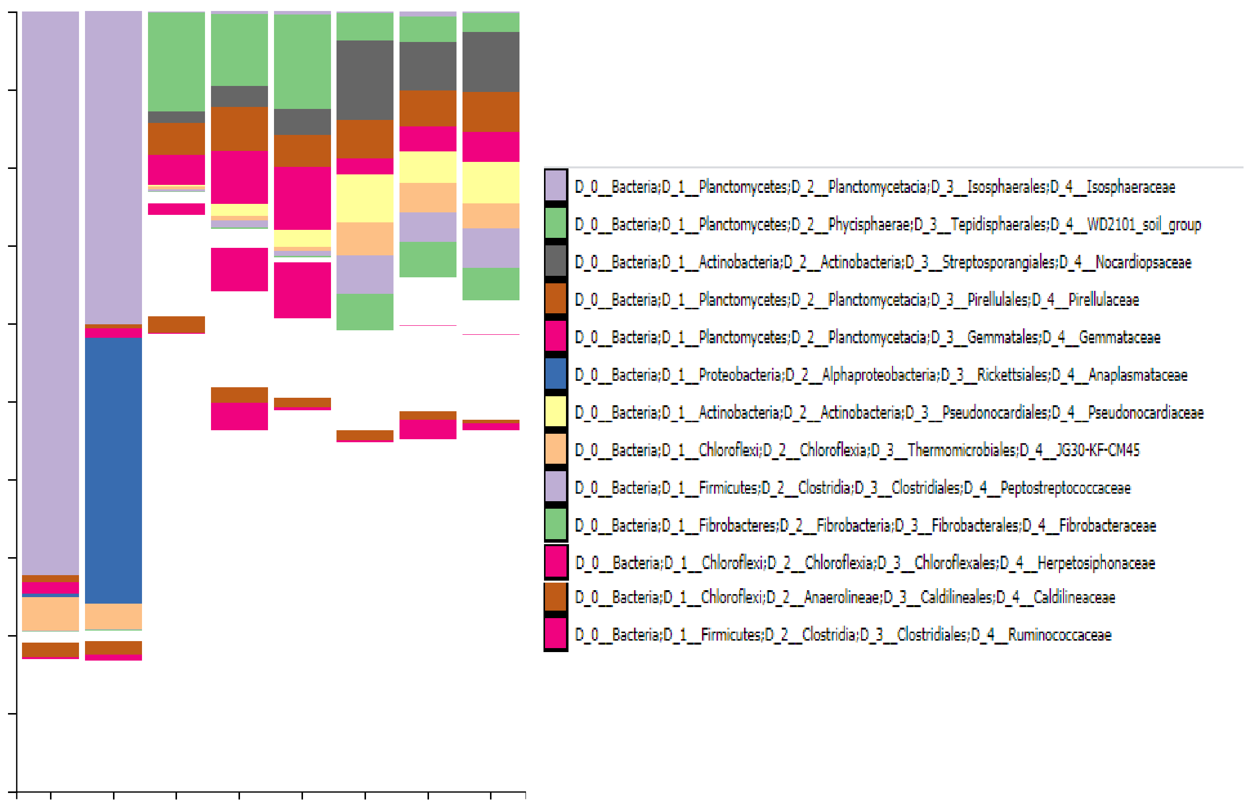

Fig. 4.5 The taxonomic affiliation at family level of the bioponic system the water from bioponics system before the planting (Biofilter), the root and the compost from the chicken manure after havesting (Soil)

The bar chart (Fig. 4.5) shows the varieties of microbial communities. The water from bioponic system before the planting, the root of lettuce and the compost of chicken manure after harvesting have the difference and similarity of the microbial diversities. The microbial communities that reported in the bar chart had links with the phosphorus transformation in bioponic systems.

This study found the varieties of bacterial families. The dominant bacterial families in the water (from bioponic system before the planting, inoculum) were *Isosphaeraceae, Anaplasmataceae, JG30-KF-CM45, Caldilineaceae* and *Xanthobacteraceae.* The Shannon index (Fig. 4.4) presented that inloculum water have lower microbial diversity than the root and the compost. The dominant bacterial families in root of lettuce were *WD2101_soil_group, Nocardiopsaceae, Pirellulaceae, Gemmataceae* and *Herpetosiphonaceae*. The dominant bacterial families in the chicken manure compost after harvesting were *Nocardiopsaceae, Pirellulaceae, Pseudonocardiaceae, Peptostreptococcaceae* and *Fibrobacteraceae.* The Shannon index (Fig. 4.4) presented that root of lettuce had similar microbial diversity as the compost of chicken manure. However, The graph (Fig. 4.3) can illustrates that the water from bioponics system before the planting (biofilter2 and biofilter3), the root of the lettuce (SM9-4-R1root, SM9-5-R2root and SM9-6-3root) and the compost from the chicken manure after havesting (SM9-1-C1soil, SM9-2-C2soil and SM9-3-C3soil) have different stucture of microbial communities from the each other.

Other study on microbial diversity in aquaponic system reported that the *Proteobacteria* were the dominant phylum of bacteria in the water and the plant roots of aquaponic system. It was found the *Actinobacteria* that had ability to transformed the phosphorus to the bioavailable form only 10% in the water (Schmautz et al., 2017). By comparison, this experiment found that the *Planctomycetes* were the dominant phylum of bacteria in the water of bioponic system. The *Proteobacteria* were the dominant phylum of bacteria in the plant root, and the *Actinobacteria* just under 35% in the water of bioponic systems. However, the other study found the plant growth promoter that were the heterotrophic bacteria (Schmautz et al., 2017) similar to this study.

4.4 Linking phosphorus and COD transformations to microbial community

This study found the varieties of microbial communities that are the plant growth-promoting (PGP) bacteria. The plant growth promoting bateria can increase nutrient availability by solubilization (for inorganic phosphorus) and mineralization (for organic phosphorus). The several research found that the *Actinobacteria* had the abilites to solubilize phosphorus and transform the phosphorus to bioavailable form (Yadav & Yadav, 2019). This study found the *Actinobacteria* that is the most dominant bacterial phylum in the compost of the chicken manure. The *Actinobacteria* phylum of genus *Nocardiopsis* spp. was one of the most dominant genera of bacteria found in the root of lettuce and the compost of chicken manure. This genus can solubilize phosphorus and increase the carbon degradtion efficiency (Montoya, Ospina, & Sánchez, 2019). The *Planctomycetes Gemmata* found in this study is plant growth promoting that can produce the hormone, fixing atmospheric nitrogen and solubilizing phophorus. The *Gemmata* has reported as the dominant genus in the organic farming (Kumar, Panwar, Rana, & Joshi, 2020). The *Gemmata* were the dominant genera bacteria in the root in this study. The *WD2101_soil_group* tht were the dominant baterial families in bioponic system had report to the bacterial familes that had ability to solubilization the phosphorus to the bioavailible form (Kumar et al., 2020). Furthermore, *JG30-KF-CM45* is heterotrophic bacteria and was the dominant bacterial family in the water and the compost of chicken manure. Thus, it can increse ability of carbon degradation (Xu, Te, He, & Gin, 2018). The *JG30-KF-CM45* was the dominant bacterial family in the water from bioponic system. Thus, the bacteria can degrade the organic carbon that presented in the COD concentration. Overall summary of link in microbial community and phosphorus and COD concentrations is shown by Fig. 4.6.

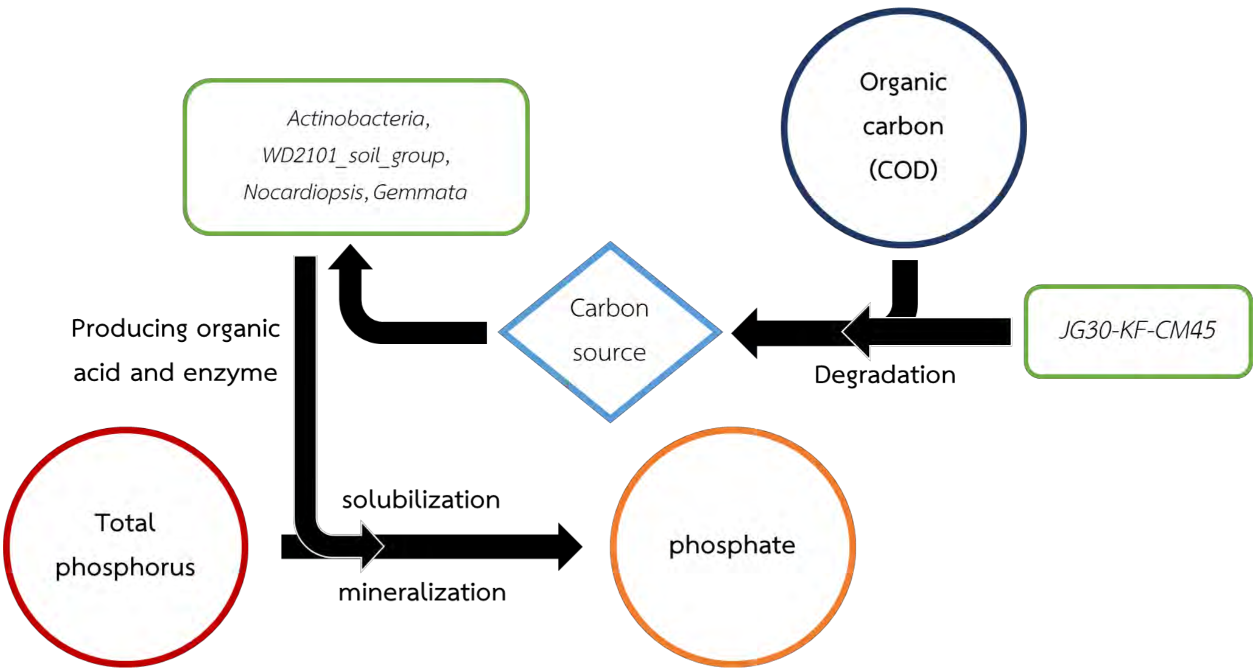

Figure 4.6 Linking of the microbial abundances to phosphorus and COD availabilities.

CHAPTER 5

RESEARCH CONCLUSIONS

5.1 Conclusions

The bioponic systems that used the chicken manure had potential for planting the lettuce. The plant had the suitable weight, and the phosphorus concentration was sufficient for the planting. From all of the experiments, the chicken manure loading rate = 500 g (phase 2) had the highest phosphorus use efficiency (23.2%) and the plant dry weight (112.8 g/18 plants). In addition, the experiment found that the amount of chicken manure loading (250 g – 500 g) did not affect the total phosphorus concentration, the COD concentration and temperature in a long term. By statistical analysis, the COD concentration and the phosphate concentration had the medium positive correlation (r=0.7177). The COD concentration and the TP concentration also had the medium positive correlation (r=0.6984). The experiment found the *Actinobacteria* (baterial phylum), *WD2101_soil_group* (bacterial family), *Nocardiopsis*, *Gemmata* that were the dominant bacterial genera in bioponic system and had the abilites to solubilize phosphorus and transform the phosphorus to bioavailable form in the water of bioponic system. Futhermore, the experiment found *JG30-KF-CM45*, heterotrophic bacteria. The *JG30-KF-CM45* was the dominant bacterial family in the water and the compost in chicken manure. It can degrade the organic carbon that presented in the COD concentration.

5.2 Research Suggestions

1. Bioponic systems should be studied with different organic waste composts to find the type of organic waste compost that is the best performance for plant growth and nutrient release.
2. Bioponic systems should be studied in various amounts of the chicken manure loading to find the optimal chicken manure loading.
3. Bioponic systems should be studied with pathogenic bacteria to be confident that the vegetables in bioponic systems were free from pathogens, safe, and hygienic.
4. Bioponic systems should be studied in economics.

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