Doctoral Thesis

Characterization of Waste Activated Sludge Derived from Livestock Wastewater Treatment and its Potential Utilization

가축폐수처리 및 잠재적 이용에 따른 폐기물 활성 슬러지의 특성

February 2019

Graduate School of Agricultural Biotechnology

Seoul National University

Sartika Indah Amalia Sudiarto

Characterization of Waste Activated Sludge Derived from Livestock Wastewater Treatment and its Potential Utilization

Thesis Advisor Professor, Cheorun Jo

Submitting a Ph.D. Dissertation of Agricultural Biotechnology

February 2019

Graduate School of Agricultural Biotechnology
Seoul National University

Sartika Indah Amalia Sudiarto

Confirming the Ph.D. dissertation written by
Sartika Indah Amalia Sudiarto
February 2019

Chairman	**Cheol-Heui Yun** (signature)
Vice Chairman	**Cheorun Jo** (signature)
Committee Member	**Myunggi Baik** (signature)
Committee Member	**Hong-Lim Choi** (signature)
Committee Member	**Dong-Yoon Choi** (signature)

ABSTRACT

Characterization of Waste Activated Sludge Derived from Livestock Wastewater Treatment and its Potential Utilization

Sartika Indah Amalia Sudiarto
Department of Agricultural Biotechnology
The Graduate School
Seoul National University

Activated sludge process is one of most common wastewater treatment process used to treat municipal, livestock, and industrial wastewater. Activated sludge process is aerobic biological wastewater treatment in which microbial aggregates are suspended in the reactor. During the wastewater treatment process of swine slurry, sludge (mostly microbial aggregates) is generated. Some of the sludge are recycled back to the wastewater treatment system to maintain the system while most of the generated sludge are waste. The waste activated sludge (WAS) should be treated to avoid further environmental contamination.

Conventionally, waste activated sludge managed by incineration,

landfill, land application and or ocean disposal. However, based on 1996 London Convention, ocean dumping of sewage sludge, food waste leachates, and livestock wastewater banned since 2012-2013 in South Korea. This policy results that those types of waste should further treated and or recycled. Therefore, waste activated sludge treatment and utilization is become important area of study.

The study consists of two parts. The first part studied on the physicochemical characteristics and effect on seed germination of WAS and membrane bio-reactor effluent (MBRE). WAS and MBRE are products from swine wastewater treatment process after separation and sedimentation. Seed germination assay was performed using radish and wheat seeds to evaluate the phytotoxicity of WAS and MBRE. Germination Index (GI) then determined from seed germination assay based on root elongation and number of seed germinated when exposed to different concentration of WAS and MBRE. From the result of the study, the potential utilization of WAS and MBRE for land application is discussed. The second part of the study focused on utilization of WAS as anaerobic digestion feedstock through bio-methane potential (BMP) assay. In addition, co-digestion of WAS with swine slurry (SS), water lily (WL) biomass, or lotus (LT) biomass were also investigated.

Based on the first study, WAS and MBRE are biologically more stable than raw swine wastewater based on its biochemical oxygen demand (BOD_5) value. BOD_5 of WAS and MBRE are 804 and 376, respectively which are much less than raw swine slurry with BOD_5 of 20,563. Waste activated sludge also contains macro-nutrients (N, P, K) and micro-nutrients (Na, Mg, Fe, Ca, Cu, Zn, and Mo) that is beneficial for plant. However, WAS contains 357 and 1,589 mg/kg dry matter of Cu and Zn respectively that should be considered for land application purposes. Cu concentration is very close to the limit value for fertilizer standard, meanwhile the Zn concentration is much higher than the limit by Korean Ministry of Agriculture. Compared with MBRE, the Cu and Zn concentration is far less than the limit value (0.03 and 0.14 mg/L). Electrical conductivity (EC) of WAS and MBRE are 4.9 mS/cm and 2.4 mS/cm, respectively which appear to be high. EC is related with salinity. High EC WAS and MBRE might contribute to the secondary salinization of agricultural land when it applied for long term period. Germination index (GI) value >80% considered to be non-phytotoxic while <50% is considered to be highly toxic and not suitable for agricultural purposes. Based on the seed germination assay, WAS exhibit phytotoxicity to radish start at 5% concentration. As for MBRE, the phytotoxicity was observed at 40% concentration. In case of wheat, WAS and MBRE shows phytotoxicity at 100% concentration.

Land application of WAS is one option of WAS potential utilization. Beside land application, anaerobic digestion is also regarded as sustainable option of WAS management. Through anaerobic digestion, renewable energy in form of methane (CH_4) gas can be obtained. In this second study, batch BMP assay was conducted. Co-digestion of WAS with swine slurry (SS) and plant biomass were also investigated. WAS contains less organic matter which may reduce its potential to produce energy. Therefore, co-digestion with higher organic matter substrates is one method to increase the biogas potential.

The bio-methane potential value of waste activated sludge are range from 255.2 – 468.9 NmL CH_4/g VS-added. Swine slurry (SS), water lily (WL), and lotus (LT) plant biomass contains higher organic matter compared with WAS. Co-digestion of waste activated sludge with swine slurry, water lily, or lotus shoot biomass produce synergistic effect with $\alpha > 1$ indicating that co-digestion improve the methane potential yield. The maximum cumulative methane yield of substrate co-digestion is more than 500 NmL CH_4/g VS-added in all substrates. The increase of methane yield of co-digestion of WAS with SS, WL, and LT are 14.89, 10.97, and 16.89% respectively.

WAS and MBRE contain nutrients beneficial for plant growth such as nitrogen, phosphorus, potassium, and micronutrients. However, the application of WAS also pose environmental risk therefore, the application rate and method should be carefully designed for land application purposes of waste activated sludge (WAS). Anaerobic digestion of waste activated sludge produce renewable energy as products which can be converted into electricity. Increase of methane yield was observed on co-digestion with SS, WL, and LT. Therefore, to increase the methane yield of WAS anaerobic digestion, WAS co-digestion with higher organic matter substrates is recommended.

Keywords: *Waste activated sludge (WAS), swine wastewater, seed germination, land application, bio-methane potential, plant biomass, co-digestion*

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS AND NOMENCLATURE

Abbreviations

ADF	acid detergent fiber
ADL	acid detergent lignin
BMP	bio-methane potential
BOD	biochemical oxygen demand
BOD_5	biochemical oxygen demand 5 days
C	carbon
Ca	calcium
CMP	calculated methane potential
COD_{cr}	chemical oxygen demand chromate
Cu	copper
DM	dry matter
DM	dry matter
DO	dissolved oxygen
DW	dry weight
EC	electrical conductivity
EMY	experimental cumulative methane yield
Fe	iron
FM	fresh matter

FS	fixed solid
FW	fresh weight
GI	germination index
H	hydrogen
HHV	higher heating value
K	potassium
LT	lotus
LTAS	lotus- waste activated sludge
MBR	membrane bioreactor
MBRE	membrane bioreactor effluent
Mo	molybdenum
N	nitrogen
Na	sodium
NDF	neutral detergent fiber
O	oxygen
OM	organic matter
RL_c	mean root length of germinated seed in control
RL_s	mean root length of germinated seed in sample
RRE	relative root elongation
RSG	relative seed germination
S	sulfur
S/I	substrate/inoculum

SG_c	number of seed germinated in control
SG_s	number of seed germinated in sample
SS	swine slurry
SSAS	swine slurry- waste activated sludge
STP	standard temperature and pressure, 1 atm 273 K
TAN	total ammonia nitrogen
TMP	theoretical methane potential
TN	total nitrogen
TP	total phosphorus
TS	total solid
VS	volatile solid
WAS	waste activated sludge
WL	water lily
WLAS	water lily- waste activated sludge
Zn	zinc

Nomenclature

D_{deg}	degradability
λ	lag-phase
α	synergistic effect
R_{max}	maximum methane production rate
G_0	maximum methane yield

CHAPTER 1. GENERAL INTRODUCTION

1.1. Introduction

1.1.1. Activated sludge process

Activated sludge process is aerobic biological wastewater treatment in which microbial aggregates are suspended in the reactor. Activated sludge process was named by Arden and Lockett (1994). The name of activated sludge process was based on the main process in this system which is microorganisms is produced during the treatment process and are actively stabilized organic matter aerobically (Tchobanoglous et al., 2003). The active microbial aggregates mentioned in this system is in the form of sludge. Activated sludge process is one of the most common wastewater treatment process used to treat municipal, livestock, and industrial wastewater.

In activated sludge system, aerobic process is the main mechanism involved. The organic matter in wastewater is broken down, while microbial cell and gas compounds are the product of organic matter decomposition inside the reactor. There are 3 different mechanisms occur during the activated sludge systems such as:

1. Organic matter (OM) conversion

$$\text{Organic matter} + O_2 \rightarrow CO_2 + NH_3 + H_2O$$

2. Cell synthesis

$$\text{Organic matter} + O_2 \rightarrow \text{New microbial cells}$$

3. Microbial cell degradation:

$$\text{Microbial cells} + O_2 \rightarrow CO_2 + NH_3 + H_2O$$

Aerobic process needs to ensure adequate oxygen (O_2) supply throughout the organic matter. Inadequate O_2 supply may result in system shift to anaerobic process that may greatly reduce system efficiency. O_2 availability is very important in aerobic process that it becomes the limiting factor. Addition of oxygen inside the aerobic wastewater treatment process often necessary to completely break down the organic matter of pollutant and to ensure proper contact between activated sludge and the substrate (mixing purpose).

There are at least three different mechanisms occurs in activated sludge process which are biological, chemical, and physical process. Physical process includes pre-treatment and post-treatment of wastewater. Filtration and separation of coarse material are the examples of wastewater pre-treatment while sludge settlement and drying are the example of the post-treatment in activated sludge system. Both pre- and post-treatment do not occur in the main reactor. Only chemical and biological process occur in the main reactor. As explained before, biological process includes microbial cell growth and nutrient assimilation and chemical process includes the conversion of organic or inorganic matter into another form such as organic matter into CO_2 and NH_4^+ or NH_4^+ to NO_3^-.

In the activated sludge process, nutrient removal by microorganisms also occurs. Major nutrient removal happens in activated sludge process are nitrogen and phosphorus removal. Biological nitrogen removal involves two different pathways, nitrification and denitrification. In nitrification process, ammonium nitrogen (NH_4^+) is oxidized into nitrate (NO_3^-). So, nitrification process aimed to remove NH_4^+ from the wastewater. While denitrification process purpose is to remove NO_3^- produced from the nitrification process. As a result, the nitrogen (N_2) in the wastewater is removed.

Nitrification is oxidation of NH_4^+ into NO_3^- under strict aerobic condition. It is accomplished by a group of autotroph chemolithotropic bacteria called nitrifying bacteria. Nitrifying bacteria can be found in water and soil (Madigan et al., 2012). It plays an important role in the environmental nitrogen cycle. It also consists of different group based on their metabolism. One group can oxidize NH_4^+ into NO_2^- and another group oxidized NO_2^- into NO_3^-. Nitrification is a two-step process. The chemical reaction of nitrification is:

- NH_4^+ oxidation

$$\frac{1}{6}NH_4^+ + \frac{1}{4}O_2 \rightarrow \frac{1}{6}NO_2^- + \frac{1}{3}H^+ + \frac{1}{6}H_2\mathrm{O}$$

- NO_2^- oxidation

$$\frac{1}{2}NO_2^- + \frac{1}{4}O_2 \rightarrow \frac{1}{2}NO_3^-$$

Denitrification is biological NO_3^- reduction process which produced gaseous nitrogen (N_2) which result in loss or removal of nitrogen from the wastewater. The N_2 then escape into the atmosphere and may undergo nitrogen cycle in the environment. Denitrification process is important in wastewater treatment since it is the pathway to remove nitrogen from the wastewater. The nitrification process needs to be followed by denitrification process to remove nitrogen because nitrification process only converts NH_4^+ to NO_3^- which will be converted into N_2 in the denitrification process.

Mostly, denitrification process is performed under anoxic condition in the presence of denitrifying-heterotrophic bacteria. NO_3^- is act as an electron acceptor instead of O_2 and organic carbon as the energy source (Ahn, 2006). However, inorganic carbon can also be used as an electron donor by denitrifying bacteria (Rittman and McCarty, 2001). The main characteristic of denitrification reactor in activated sludge system is the minimum aeration.

Phosphorus removal using activated-sludge systems includes the combination of aerobic and anaerobic process. Activated-sludge system for phosphorus removal usually called biological phosphorus removal (BPR) or enhanced biological phosphorus removal (EBPR). The main mechanism for phosphorus removal in EBPR is accumulation of P into bacterial cells in the form of polyphosphate (polyP) granules (Seviour et al., 2003). The microbes' uptake phosphorus and store polyP in the cell and known as polyP accumulating organism (PAO) (Seviour et al., 2003) or polyphosphate accumulating bacteria (PAB) (Mino, 2000). The phosphorus is removed from the wastewater along with the removal of sludge containing PAO (Seviour et al., 2003). This microorganism requires specific condition that encourages the P uptake and accumulation. Polyphosphate accumulating organisms have high phosphorus content in their biomass. It can be up to 12% of dry biomass while only 3% in non-PAO biomass (Nielsen et al., 2012). High amount of polyphosphate residue in the microbial cell is an indicator that the microbes live in high concentration of phosphorus medium (Kulaev and Kulakovskaya, 2000). In the wastewater, phosphorus is available in different forms. Phosphorus forms in aqueous solution are available as:

1. Orthophosphate

This form is available for metabolisms. The form of orthophosphate is PO_4^{3-}, HPO_4^{2-}, H_2PO^{4-}, and H_3PO_4.

2. Polyphosphate

Is molecules that consist of 2 or more phosphates in the form of polymer. The hydrolysis of polyphosphate result in orthophosphate molecules.

3. Organic phosphate

Organic phosphate is phosphate that bound in organic compounds. This form is main concern in industrial waste and wastewater sludge.

During the activated sludge process, microbial cell growth will be taken place. The biomass yield is the amount of biomass produced per substrate utilization. The bacterial growth is measured as synthesis yield, which is the production of bacterial biomass upon substrate consumption. Different bacteria and growth condition will result in different synthesis yield as depicted in Table 1.1.

Synthesis yield gives an idea of bacterial growth, however in full-scale wastewater treatment, it is difficult to differentiate between the bacterial biomass and other components such as solid particles. One of the parameters in wastewater treatment is solid content. Solid contents of the wastewater include biological-derived solid and non-biological solid.

Table 1.1. Typical bacteria synthesis yield coefficients for common biological reactions in wastewater treatment (Tchobanoglous et al., 2003).

Growth condition	Electron Donor	Electron acceptor	Synthesis yield
Aerobic	Organic compounds	Oxygen	0.4 g VSS/g COD
Aerobic	Ammonia	Oxygen	0.12 g VSS/g NH_4-N
Anoxic	Organic compounds	Nitrate	0.30 g VSS/g COD
Anaerobic	Organic compounds	Organic compound	0.06 g VSS/g COD
Anaerobic	Acetate	Carbon dioxide	0.05 g VSS/g COD

1.1.2. Waste activated sludge production from swine farming

In 2008, about 2,966,000 and 6,514,000 dry metric ton sewage sludge produced from wastewater treatment plants in China and USA respectively (Yin et al., 2016). However, sludge production data from livestock industry is still not available.

Based on FAO statistical database, the total number of swine in South Korea is about 10,100,000 head in 2014. Assume that all the waste produces by the pig is as pig slurry and slurry production equal to 5 L/day/head, it will be 51,510,000 L/day of swine slurry are produced. The average COD of swine slurry is 57,000 mg/L according to Suresh and Choi (2011). This means, with those amount of slurry, total COD would be 2,936,070,000 g COD/d. Based on table 1.1, synthesis yield of aerobic condition with organic compounds as electron donor is 0.4 g VSS/g COD. Assume that all the swine slurry is treated aerobically and VSS is sludge, there will be 428,666-ton waste sludge per year, from swine farming.

1.1.3. Waste management in South Korea

In general, livestock waste management system in South Korea is presented in Figure 1.1. There is public and private livestock wastewater treatment facilities. Each of them divided into re-sourcification facilities and purification facilities. The re-sourcification facilities will be focus on processing the waste into products such as fertilizer through composting process. The waste going through re-sourcification facilities will be converted into fertilizer or soil amendment and returned into the agricultural cropland. Meanwhile, from the purification facilities, the treated waste is disposed into the environment. The purification facilities focus on livestock wastewater treatment to reach discharge permit.

Livestock wastewater contains high concentration of macro- and micro-nutrients. However, before discharging into the water bodies, the concentration of these nutrients should be below the acceptable limit and it depends on the regulation of each country. Sometimes, the primary and secondary wastewater treatments are not enough to reduce the total nutrients in the livestock wastewater. On the other hand, the nutrients of the wastewater can be utilized by photosynthetic organisms such as plant and microalgae. The plant will uptake macro- and micro-nutrient in the wastewater and reduce the nutrient in the wastewater so that can be discharged safely to the water bodies. The plant biomass can be utilized as a resource such as for biofuel and bioethanol raw feedstock or even used as feed for livestock animal. The plant biomass produced from tertiary wastewater treatment might be utilized in integration with waste activated sludge utilization as bioenergy feedstock.

1.1.4. Waste activated sludge composition

Activated-sludge system as implied by the name, focus on the sludge, which is microbial aggregates. The sludge is partly waste and recycled back into the system as inoculum for maintaining the performance. This recycled sludge contains microorganism that responsible for processing the fresh wastewater. In most cases, the activated-sludge can reduce the performance of the system, when excessive sludge is accumulated in the system. Therefore, most of the sludge should be discarded or going through tertiary treatment.

Microbes are important in activated-sludge wastewater treatment system. Microbes are the key part of organic material decomposition in the system. Microbes also able to utilize some inorganic nutrients, thus reduced the inorganic compounds concentration such as NH_4^+ or phosphorus in wastewater. During the treatment process, microbes produced soluble microbial products (SMP) that recently become researcher's interest. This because SMP comprises major soluble organic matter in the effluent so that will limit the system performance (Barker and Stuckey, 1999).

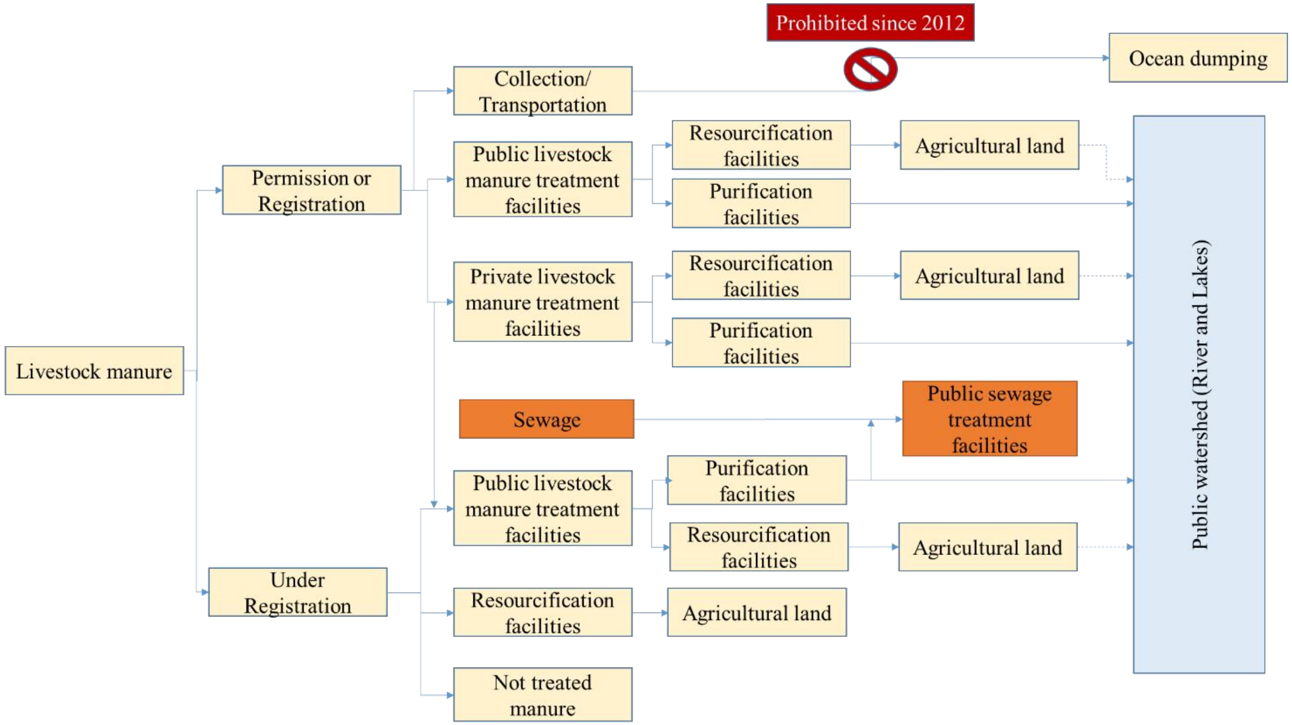

Figure 1.1. Framework of livestock waste treatment system in South Korea (Jeong et al., 2013)

Microbes in the activated-sludge system are varied. It depends on the waste characteristics, system type, operational management, and environmental factors. The microbial community in activated sludge system form aggregates or flocs. Aeration or mixing in the reactor causes the aggregates distributed evenly and suspended in the wastewater. The microbial community feeds upon organic matter provided in the reactor. Prokaryotes (bacteria) and microscopic eukaryotes such as protozoa, crustacean, nematodes, and rotifers are available in the activated sludge system. It is even possible to find virus (phage) in the activated sludge system. The microbial community in the activated sludge system consists of primary and secondary consumers. Primary consumers feed on organic particles while the secondary consumers feed on materials that is released from primary consumers (Rittmann and McCarty, 2001).

The activated sludge flocs mainly comprise of bacteria and both organic and inorganic material. Its size varies between <1μm and ≥1000 μm (Bitton, 2011). The flocs can be monitored regularly through microscopic observation Even though flocs size is small, there still an oxygen zonation. The outer part of the flocs is the aerobic zone while the core of the flocs is the anoxic or even anaerobic zone. It is possible since flocs formation may limit oxygen diffusion into its core part, thus creating anoxic zone.

Activated sludge flocs contain microbial cells, organic compounds, bound water, microbial products such as EPS and SMP, and other compounds. EPS is divided into loosely bound EPS and tightly-bound EPS meanwhile for SMP, it consists of Utilization Associated Products (UAP) and Biomass Associated Products (BAP). ES produced by microbes to form aggregates in the activated sludge. EPS is located outside the cell surface. EPS might contain protein, carbohydrates, lipids, and other compounds such as humic acids.

In biological wastewater treatment systems, most of the microorganisms are present in the form of microbial aggregates such as sludge flocs, biofilms, and granules. EPS are present both outside of cells and in the interior of microbial aggregates. EPS are mainly the high-molecular-weight secretions from microorganisms, the products of cellular lysis, and hydrolysis of macromolecules. In addition, some organic matters from wastewater can also be adsorbed to the EPS matrix. The form of EPS that exist outside the cells can be subdivided into bound EPS and soluble EPS. Generally, those two types of EPS can be separated by centrifugation (Sheng et al., 2010).

Majority of organic compounds in the waste activated sludge are in the form of microbial cells. Degradation of such microbial cell is the main problems to further process or utilize waste activated sludge. The structure of cell membranes protects it from osmotic lysis. The cell walls of microbes consist of strands of glycan cross-linked by peptide chain that is hard to be biodegraded. Therefore, prior to treatment or utilization, microbial cells should be broken down to release its contents, which then will be easier to be converted into simple compounds (Weemaes and Verstraete, 1998).

Waste activated sludge contains organic matter, microbial biomass, microbial products, and other compounds. Suspended solids in activated sludge are mostly bacteria which contain 70-80% water inside the cell. In addition, even well flocculated activated sludge traps considerable amounts of bound water outside the cells. Therefore, the activated sludge floc is only slightly heavier than the water in which it is suspended. The difference in density is typically only about 0.0015 g/cm^3 (Rittman and McCarty, 2001).

Improvement in organic matter, nutrient contents, soil porosity, bulk density, aggregate stability, and water holding capacity as well as enhancement of microbial biomass and its nutrient mineralization potential has been reported (Banerjee et al., 1997; Singh and Agrawal, 2008; Clarke and Smith, 2011). Meanwhile, the accumulation of undesirable substances contained in the sludge is an important factor to consider within sludge management, as these substances might reach the food chain. Heavy metals, pathogens, and organic pollutants can also have adverse effect on soil functioning and biodiversity.

Most of the research regarding waste activated sludge is utilizing municipal and sewage-derived waste activated sludge. Fewer studies were conducted for waste activated sludge derived from livestock wastewater treatment. Most studies on livestock waste evaluate the fresh livestock waste, composted livestock waste or digested livestock waste. Livestock wastewater might exhibit different characteristics compared with WAS derived from municipal and sewage sludge.

To evaluate the potential utilization of waste activated sludge derived from livestock wastewater, characteristics study may help to understand the nature and composition of swine-slurry derived waste activated sludge. Based on the results we can determine how to manage or utilize waste activated sludge in further research. Livestock waste sludge is less researched than sewage and municipal waste activated sludge, therefore the results of this study might become a reference for further research. In addition, characteristics of WAS from livestock wastewater might be different with those from sewage and municipal wastewater.

1.1.5. Waste activated sludge management

After swine wastewater treatment process, the sludge generated should be properly treated to avoid further environmental contamination. Conventionally, waste activated sludge managed by incineration, landfill, land application and or ocean disposal. However, based on the 1996 London Convention, ocean dumping of sewage sludge, food waste leachates, and livestock wastewater banned since 2012-2013 in South Korea. Not only South Korea, but European countries also banned ocean disposal and having stricter landfill regulations. This policy results that those types of waste should further treated and or recycled. Therefore, waste activated sludge treatment and utilization becomes important area of study. In addition, some conventional management such as landfill and ocean disposal poses risk of environmental pollution. Meanwhile, land application of waste activated sludge has a limit. Incineration still one of the management practices that still applied, however, it needs high cost to establish the system. Moreover, in the future, landfilling and incineration might not be suitable anymore since the less land available and strict regulation for the environment (Zhang et al., 2010).

Waste activated sludge management going towards utilization of WAS for different purpose according to the needs, available technology, and economic benefit of wastewater treatment plants, the community surrounding it, and government regulation. Resource recovery from waste activated sludge become an interesting area of study. Based on review by Tyagi and Lo (2013) resource recovery from waste activated sludge can be divided into 3 routes, biochemical, thermochemical, and mechanical-chemical (Figure 1.2). In biochemical method, anaerobic digestion is the main pathway for resource recovery, integrating energy production with waste treatment.

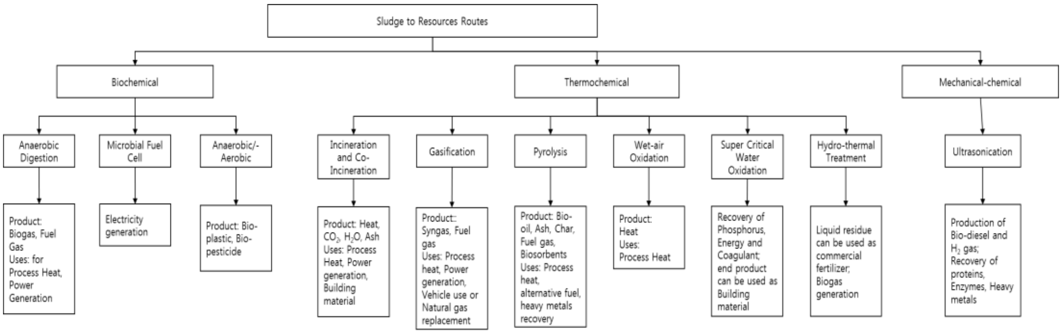

Figure 1.2. Routes of resource to recovery from waste sludge (Tyagi and Lo, 2013)

Anaerobic digestion and land application are regarded as sustainable option for waste activated sludge management. In anaerobic digestion, the microorganisms degrade the sludge and produced energy in form of CH_4 which can be converted into heat or electricity. Beside anaerobic digestion, land application is one way to utilize the effluent and waste activated sludge. In this study, the physicochemical characteristics, the effect on seed germination, and bio-methane potential of waste activated sludge were studied to evaluate the potential of waste activated sludge derived from swine slurry treatment process for land application and as bioenergy feedstock. The co-digestion of waste activated sludge with swine slurry and plant biomass were also investigated.

1.2. Objectives

1. Characterize the physicochemical characteristics of waste activated sludge derived from aerobic swine wastewater treatment process
2. Evaluate the potential utilization of waste activated sludge for land application and as bioenergy feedstock resources.

References

Ahn, Y.H. 2006. Sustainable nitrogen elimination biotechnologies: a review. *Process. Biochem.*41: 1709-1721.

Banerjee, M. R., Burton, D. L., & Depoe, S. (1997). Impact of sewage sludge application on soil biological characteristics. *Agriculture, ecosystems & environment*, *66*(3), 241-2

Barker, D.J., D.C. Stuckey. 1999. A review of soluble microbial products (SMP) in wastewater treatment system. *Wat.Res.* 33: 3063-3082.

Bitton, G. 2011. *Wastewater microbiology 4th edition.* New Jersey: Wiley-Blackwell.

Clarke, B. O., & Smith, S. R. (2011). Review of 'emerging'organic contaminants in biosolids and assessment of international research priorities for the agricultural use of biosolids. *Environment international*, *37*(1), 226-247.

Jeong, D. H., Shin, J., Lee, C., Yu, S., & Kim, Y. (2013). A study on the improvement measures of livestock manure management and organic fertilizer use in Nonsan area. *Journal of Environmental Impact*

Assessment, *22*(4), 345-359.

Kulaev, I., Kulakovskaya, T. 2000. Polyphosphate and phosphate pump. *Annu. Rev.Microbiol.*54:709-734.

Madigan, M.T., Martinko, J.M., Stahl, D.A., Clark, D.P. 2012. *Brock biology f microorganisms.* San Francisco: Benjamin Cummings.

Nielsen, P.H., Saunders, A.M., Hansen, A.A., Larsen, P., Nielsen, J.L. 2011. Microbial communities involved in enhancedbiological phosphorus removal from wastewater- a model system in environmental biotechnology. *Curr.Opin.Biotechnol.*23: 452-459.

Rao, N.N., Gomez-Garcia, M.R., Kornberg,A. 2009. Inorganic polyphosphate: essential for growth and survival. *Annurev.Biochem.*78: 605-647.

Rittmann, B.E., McCarty, P.L. 2001. *Environmental biotechnology principles and applications.* McGraw-Hill: New York.

Seviour, R.J., Mino,T., Onuki, M. 2003. The microbiology of biological phosphorus removal in activated sludge system. *FEMS microbiology review*.27:99-127.

Sheng, G.P., Yu, H.Q., Li, X.Y. 2010. Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: a review. *Biotechnol Adv*, **28**(6), 882-94.

Singh, R. P., & Agrawal, M. (2008). Potential benefits and risks of land application of sewage sludge. *Waste management*, *28*(2), 347-358.

Tchobanoglous, G., Burton, F.L., Stensel, H.D., Metcalf, Eddy. 2003. *Wastewater Engineering: Treatment and Reuse*. McGraw-Hill.

Tyagi, V.K., Lo, S.-L. 2013. Sludge: A waste or renewable source for energy and resources recovery? *Renewable and Sustainable Energy Reviews*, **25**, 708-728.

Weemaes, M.P., Verstraete, W.H. 1998. Evaluation of current wet sludge disintegration techniques. *Journal of Chemical Technology and Biotechnology*, **73**(2), 83-92.

Yin, Y., Liu, Y.-J., Meng, S.-J., Kiran, E.U., Liu, Y. 2016. Enzymatic pretreatment of activated sludge, food waste and their mixture for enhanced bioenergy recovery and waste volume reduction via anaerobic digestion. *Applied Energy*, **179**, 1131-1137.

Zhang, D., Chen, Y., Zhao, Y., Zhu, X. 2010. New sludge pretreatment method to improve methane production in waste activated sludge digestion. *Environmental science & technology*, **44**(12), 4802-4808.

CHAPTER 2. Characterization of waste activated sludge derived from swine wastewater treatment and its effect on seed germination

This chapter will be published in SCI(E) journal as partial fulfillment of Agricultural Biotechnology's Ph.D. program for Sartika Indah Amalia Sudiarto

Abstract

Waste activated sludge derived from swine wastewater treatment system eventually should be utilized. The aims of the study are to determine waste activated sludge (WAS) and effluent (MBRE) derived from swine wastewater treatment potential for land application based on its characteristics and seed germination assay. Waste activated sludge (WAS) contain 357 and 1,589 mg/kg dry matter of Cu and Zn respectively which should be considered for land application purposes. EC of both WAS and MBRE appear to be high which is 4.95 and 2.4 mS/cm. The germination assay shows that WAS phytotoxicity on radish was observed starting at 5% concentration meanwhile for wheat seed, the phytotoxicity of WAS is seen at 100% concentration. Membrane bio-reactor effluent (MBRE) phytotoxicity was observed at concentration of 40% for radish seed and 100 % for wheat seed. There is significant difference ($p<0.05$) of GI between radish and wheat in WAS treatment and starting at 20% in MBRE treatment. Radish exhibit different response compared with wheat towards WAS at every concentration and at higher concentration of MBRE. Overall, WAS and MBRE contain nutrients beneficial for plant growth such as nitrogen,

phosphorus, potassium, and micronutrients. However, the application of WAS also poses environmental risk, especially in relation with its salinity indicated by high EC. The application rate and method should be considered for land application purposes of waste activated sludge (WAS).

Keywords: *Waste activated sludge, swine wastewater, seed germination, effluent*

2.1. Introduction

Intensified livestock farming activities eventually will pollute the environment whether directly or indirectly. Pollutant from livestock waste produced during the intensive animal farming can enter the environment and contaminate air, water, and soil. Livestock waste contains organic matter, antibiotics, nutrients, heavy metals, pathogens, and naturally produced hormones that can contaminate the environment (Burkholder et al., 2007). On the brighter side, livestock waste contains macro- and micronutrients and is a great natural fertilizer for crop system after the treatment process. However large industrial farming or concentrated animal feeding operation produce excessive waste.

Among other livestock, swine contribute highest in terms of livestock waste generation in South Korea. Swine farming activity contributes about 40% waste generated from livestock industry (Lim and Kim, 2015). Therefore, tremendous amount of swine wastewater has been generated due to intensive farming activities.

Biochemical oxygen demand (BOD) of livestock wastewater is 90 times higher than domestic sewage wastewater (MOE, 2017). Livestock waste and wastewater contain high amounts of organic matter and nutrients. As an example, BOD_5 and soluble chemical oxygen demand $(COD)_{cr}$ of swine wastewater are 20,563 and 24,281 mg/L respectively, total nitrogen swine wastewater can be as high as 4,546 mg/L, and the total phosphorus content has been reported at 2,765 mg/L (Suresh and Choi, 2011). Swine wastewater also contains a considerable amount of micronutrients. Therefore, there has been massive work and policy regarding the treatment of livestock wastewater to prevent pollution to the environment.

Swine wastewater contains high organic matter and nutrient, therefore, swine wastewater treatment is necessary to prevent environmental contamination. In South Korea, livestock waste is often utilized as resources for fertilizer after composting or other treatment processes (MOE, 2017). Based on the "Act on the management and use of livestock excreta", the livestock producers should establish livestock waste treatment facilities. However, wastewater treatment process also generates sludge which should be disposed of or further treated.

Aerobic wastewater treatment of swine wastewater reduced its organic matter content as well as the BOD and COD due to the biodegradation by microorganisms, in addition, nutrient removal also occurs during the process. However, not all nutrients are removed during the process, for example, nitrogen removal pathway from the decomposition of organic matter include volatilization in which nitrogen in the form of gas is released to the atmosphere. Meanwhile, phosphorus removal does not involve volatilization, therefore, its presence remains even though the form might be changed.

Nutrients that are not volatilized during the treatment process are concentrated in the sludge fraction or present in the soluble form in the wastewater effluent. The sludge is separated from the wastewater, therefore, the effluent contains fewer nutrients. However, most of the nutrients, as well as microbial biomass remain in the waste sludge. The wastewater effluent might be utilized as irrigation water in the cropland. In addition, it is also possible that waste activated sludge (WAS) are also utilized as resources for fertilization considering waste activated sludge (WAS) are product of wastewater treatment process which contains nutrients and stabilized organic matter.

The use of sewage sludge as soil amendment is an important process in sustainable sludge management practices. From the study by Alvarenga et al. (2015), the benefit of land application of sewage sludge is contributed by its high organic matter and nutrient content (N, P, and K). However, high ammonium content might contribute to ammonia emission from land after application. Land application of sludge might contribute to nutrient cycling and improvement of soil properties. The application of sludge might as well increase the biodiversity of soil which indirectly will affect the fertility of the soil. Sludge application on land is a common practice in sludge management. Proper application of sewage sludge as soil amendment improve the soil properties (Roig et al, 2012).

Land application is one way to utilize the effluent and waste activated sludge. The effluent from swine wastewater treatment process also has potential to be utilized as irrigation water. The land application of effluent and waste activated sludge purpose is for plant growth. Therefore, its effect on plant should be assessed. Untreated swine slurry or manure are known to release phytotoxic substances which will inhibit plant growth. Phytotoxicity is delay or inhibition of seed germination or plant growth or any other adverse effect caused by phytotoxin. Waste activated sludge and wastewater effluent might also exhibit the properties since it is derived from the swine slurry. However, the degree of its toxicity might be different. In the case of land application of wastewater effluent and waste activated sludge, the phytotoxicity assay is important. Utilization of sludge as a soil amendment or fertilizer might affect the growth, development, and physiology of plants. Some study reported that the application of sewage sludge changes biochemical change in plants (Wyrwicka and Urbaniak, 2016).

Seed germination assay has been known to evaluate the maturity of compost. Seed germination assay also used to evaluate the phytotoxicity of wastewater. The germination assay is low cost and simple. The recommended seed for germination assay according to USEPA and USFDA are cucumber, lettuce, radish, red clover, and wheat (Priac et al, 2013). Different species might have different sensitivity (Priac et al, 2013). Seed germination is critical step in agricultural practices which is needed to ensure the crop productivity. Wastewater contains high amount of nutrients, organic matter, and heavy metals which might affect seed germination and further plant growth and development.

At the end of aerobic treatment system of swine wastewater treatment process, the treated wastewater is separated into wastewater effluent and waste activated sludge. Waste activated sludge contains higher organic matter, nutrients contents, and microbial biomass. The characteristics of the effluent and waste activated sludge are different. Therefore, its effects on seed germination are likely to differ. In this study, the characteristics of waste activated sludge and effluent are determined and the effect of treated wastewater effluent and waste activated sludge are evaluated.

The sensitivity of different type of seed may influence its response towards waste activated sludge and treated wastewater. Therefore, in this study, radish and wheat seeds are used. Different responses between two types of seeds were also compared. It is expected that germination assay will give information on waste activated sludge potential and treated wastewater effluent to be used for land application into agricultural field.

2.2. Objectives

1. Determine waste activated sludge and wastewater effluent derived from swine wastewater treatment characteristics and its potential for land application
2. Evaluate the effect of waste activated sludge and treated wastewater on radish and wheat seed germination.

2.3. Materials and Method

2.3.1 Waste activated sludge and treated wastewater samples

Waste activated sludge (WAS) and treated wastewater samples are obtained from small-scale swine wastewater treatment process treating about 90 L swine slurry per days, located at Hoengseong. The treatment process consists of anoxic and oxic reactors, sedimentation tank, and membrane filtration tank (Figure 2.1). The waste activated sludge was obtained from the sedimentation tank. While the treated wastewater was taken from the membrane bio-reactor and labeled as membrane-bioreactor effluent (MBRE). The samples were taken on July and August 2018.

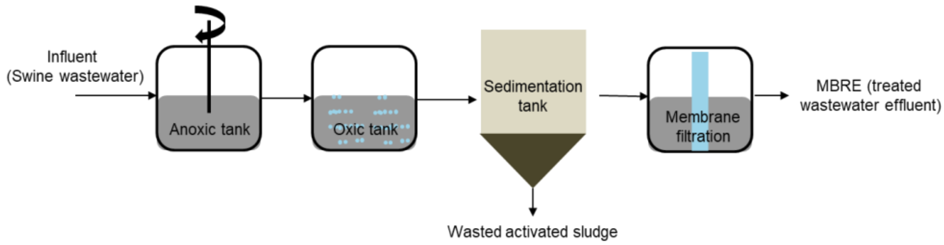

Figure 2.1. Schematic view of swine wastewater treatment system

2.3.2 Physicochemical characteristics measurement

The dissolved oxygen (DO) measured by using DO meter (YSI 500), electrical conductivity (EC) were measured by using conductivity meter (Hanna Instrument, Ltd., Italy) and pH levels were measured by pH meter (Trans Instrument). Total nitrogen (TN), total ammonia nitrogen (TAN), nitrate (NO_3^-N), nitrite (NO_2^-N) and total phosphorus (TP) were determined by the persulfate digestion method, salicylate method, cadmium reduction method, diazotization method, and molybdovanadate method with acid persulfate digestion, respectively, with Hach chemical reagents according to the manufacturer protocols (DR3000, Hach, USA).

2.3.3 Total solid, volatile solid, and fixed solid

Total solid (TS), volatile solid (VS) and fixed solid (FS) of WAS and MBRE were determined according to APHA standard method 2450 (APHA, 2005). About 10 mL well-mixed sample of waste activated sludge or wastewater effluent were weighed and dried in an oven at 103-105 ˚C, then the total solid was calculated and expressed as mg/L fresh weight or % weight. After total solid measurement, the residue was ignited in muffle furnace at temperature of 550 ˚C to determine the volatile and fixed solid. The volatile and fixed solid were calculated and expressed as mg/L fresh weight or % dried weight.

2.3.4 Biological and chemical oxygen demand analysis

Biological oxygen demand was measured as 5-day BOD (BOD_5). The procedure consists of filling with diluted and seeded sample, to overflow, 300 ml of BOD bottles and incubating it at 20°C for 5 days. Dissolved oxygen is measured at initial and after the incubation period. The BOD is calculated from the difference between initial and final dissolved oxygen multiplied by the dilution factor. The BOD_5 determined by calculation using formula (1):

$$BOD_5 = \frac{(D_1 - D_2) - (S)V_S}{P} \tag{1}$$

where:

D_1 = Dissolved oxygen of diluted sample immediately after preparation, mg/L

D_2 = Dissolved oxygen of diluted sample after 5 d incubation at 20 °C, mg/L

S = Oxygen uptake of seed, Δ DO/mL seed suspension added per bottle, S= 0 if the samples are not seeded.

V_s = Volume of seed in the respective bottle, mL

P = Decimal volumetric fraction of sample used; 1/P = dilution factor

Seed is not used for BOD_5 analysis since the sample already contains microorganism. Therefore (S) Vs equal to 0.

2.3.5 Germination Index Assay

Germination index was performed with waste activated sludge (WAS) and membrane bio-reactor effluent (MBRE) dilution series. WAS and MBRE were diluted with distilled water to obtain concentration of 5, 10, 20, 30, 40, 50, and 100%. Commercial radish and wheat seed were used in this study. Ten radish or wheat seeds were evenly distributed on filter paper in petri dishes in which 10 mL of diluted samples were added. The controlled plates were added with 10 mL of distilled water. All experiments were conducted in triplicates. The plates were then incubated at 25°C for 3 days (radish) and 6 days (wheat). After the incubation, the number of seed germinated, root length, and shoot length were measured. Relative seed germination (RSG), relative root elongation (REE), and germination index (GI) were measured using formula 2 – 4.

$$\mathrm{GI} = \frac{SG_S}{SG_C} \times \frac{RL_S}{RL_C} \times 100 \qquad (2)$$

$$\mathrm{RSG} = \frac{SG_S}{SG_C} \times 100 \qquad (3)$$

$$RRE = \frac{RL_s}{RL_c} \times 100 \quad (4)$$

where:

GI = Germination Index

RSG = Relative Seed Germination, %

RRE = Relative Root Elongation, %

SG_s = Number of seed germinated in sample, %

SG_c = Number of seed germinated in control, %

RL_s = Mean root length of germinated seed in sample, %

RL_c = Mean root length of germinated seed in control, %

2.3.6 Minerals and HHV analysis

To determine minerals, samples were dried and pre-treated then analyzed using inductively coupled plasma (ICP) atomic emission spectroscopy (AES) (ICPS-7510; Shimadzu Corp., Kyoto, Japan). Dried samples were mixed using a steel blade and screened through 1 mm mesh screen. The samples were weight about 0.5-1.0 gr then pelletized manually using Parr pelleting equipment and analyzed with Parr bomb calorimeter (Model 1341 plain jacket calorimeter, Parr Instrument). Benzoic acid pellets (3415, Parr Instrument) were utilized to standardize the oxygen bomb calorimeter prior to the analysis.

2.3.7 Statistical analysis

The analysis of variance (ANOVA) and Tukey test were used to determine the differences among treatments. Confidence interval of 95% was used for data analysis. P-value of 0.05 was utilized for all test. All statistical analysis was done with Real Statistics Data Analysis Tool in Microsoft Excel 2016.

2.4. Results and Discussions

2.4.1. Waste activated sludge and treated wastewater characteristics

Waste activated sludge and treated wastewater characteristics are presented in Table 2.1. Most of the nutrients are abundant in the waste activated sludge since most solid part of aerobically treated wastewater are saturated in the sludge. Treated wastewater mostly contain soluble nutrients. Waste activated sludge contains high total COD_{cr} with average concentration of 43,900 mg/L. However, the BOD_5 of waste activated sludge are 804 less than raw swine slurry with BOD_5 of 20,563 (Suresh and Choi, 2011) which means that waste activated sludge are more stabilized biologically.

Based on the pH value, the waste activated sludge and treated wastewater are slightly alkaline within the range of 7.28 – 7.83. The dissolved oxygen value of treated wastewater is 3.9 mg/L meanwhile waste activated sludge is 0.31 mg/L. The difference occurred because sludge was taken from the sedimentation tank and the condition promote anaerobic condition results in low dissolved oxygen concentration.

Salinity is one factor that should be considered following the land application of waste activated sludge. The salinity and EC (electrical conductivity) are related. The salinity of material applied into cropland may have long time effect especially when it is applied frequently and in long-term. High salinity wastewater or sludge might contribute to the secondary salinization on the agricultural land (Alvarenga et al., 2015). Increase in salinity will lead to phytotoxic effects on seed germination and growth (Gao et al., 2010). Land irrigation with high EC wastewater increase the soil salinity (Kiziloglu et al., 2008).

Table 2.1. Waste Activated Sludge (WAS) and Membrane Bioreactor Effluent (MBRE) characteristics

Parameters	WAS	MBRE
pH	7.28 ± 0.41	7.73 ± 0.26
Electrical Conductivity (EC) (mS/cm)	4.95 ± 1.69	2.4 ± 4.61
Dissolved Oxygen (DO) (mg/L)	0.31 ± 0.39	3.39 ± 0.66
Total Nitrogen (TN) (mg/L)	2,425 ± 403	197.5 ± 41.13
Total Ammonia Nitrogen (TAN) (mg/L)	882.5 ± 284.06	206.25 ± 97.93
NO_2^--N (mg/L)	20.80 ± 9.47	97.75 ± 6.75
NO_3^- -N(mg/L)	50.00 ± 28.28	325 ± 25.17
Total Phosphorus (TP) (mg/L)	7,896 ± 1,000	63.67 ± 27.28
Chemical Oxygen Demand (COD_{cr}) (mg/L)	43,900 ± 7,495	1,450 ± 132.29
Biochemical Oxygen Demand 5-day (BOD_5) (mg/L)	704.33 ± 297.16	361.9 ± 132.76
Total Solid (TS) (% FM)	5.67 ± 1.48	0.36 ± 0.15
Volatile Solid (VS) (%FM)	3.35 ± 1.17	0.15 ± 0.04
Fixed Solid (FS) (%FM)	2.32 ± 0.44	0.21 ± 0.01
Higher Heating Value	15.42 ± 0.28	na*

(HHV) (MJ/kg DM)		
Minerals		
K (mg/kg DM)	14,632 ± 2,482	740.87 ± 35.19
Na (mg/kg DM)	1,721 ± 1,721	113.85 ± 16.26
Mg (mg/kg DM)	15,220 ± 119.15	34.80 ± 2.86
Fe (mg/kg DM)	10,519 ± 325.13	0.49 ± 0.14
Ca (mg/kg DM)	68,547 ± 2,829	39.7 ± 21.82
Cu (mg/kg DM)	395.4 ± 24.04	0.03 ± 0.08
Zn (mg/kg DM)	1,589 ± 394.25	0.14 ± 0.015
Mo (mg/kg DM)	4.16 ± 0.16	nd**

Value represented as means ± standard deviation (SD)

FM : Fresh matter; DM: Dry matter

* na : not available

**nd : not detected

Waste activated sludge contain higher EC values (Table 2.1) than treated wastewater. The EC value of waste activated sludge and treated wastewater are 4.95 and 2.4 mS/cm. The waste activated sludge EC value of 4.95 mS/cm is higher than sewage sludge which is in the range of 1.23 – 3.49 mS/cm and lower than agricultural waste compost and pig slurry digestate which is about 6.12 mS/cm and 7.95 mS/cm (Alvarenga et al., 2015). Phosphorus content is much higher in the waste activated sludge than in treated wastewater. In activated sludge system, the phosphorus is accumulated in the sludge part since it is incorporated into bacterial cells (Seviour et al., 2003). Meanwhile, phosphorus content in the treated wastewater in the soluble form and less amount.

Table 2.2. Heavy metal limits of Korea Fertilization Standard (Ravindran et al., 2017)

Heavy metals	Heavy metals limits (mg/kg) Korea fertilization standard
Copper (Cu)	360
Cadmium (Cd)	5
Chromium (Cr)	200
Nickel (Ni)	45
Mercury (Hg)	2
Lead (Pb)	130
Zinc (Zn)	900
Arsenic (As)	45

According to the Korean Fertilizer standard (Table 2.2), the limit range for Cu and Zn are 360 and 900 mg/kg dried weight. Waste activated sludge contains 357 and 1,589 mg/kg dry matter of Cu and Zn respectively. Cu concentration is very close to the limit value for fertilizer standard, meanwhile, the Zn concentration is much higher than the limit by Korean Ministry of Agriculture. Therefore, the land application of waste activated sludge might pose risks based on the Zn concentration. Direct land application of waste activated sludge in this regard might not be a good option. A strategy for application should be made to reduce the Zn load to the cropland before application. Blending the waste activated sludge with other materials might be an alternative or further treatment of waste activated sludge also an option. Mixing sludge with other materials may reduce its Zn concentration per dry matter. Kwon et al. (2009) reported that mixing vermicomposted sewage sludge with powdered oyster shell reduce the heavy metals content to the acceptable limit for sludge to be applied. Further treatment such as vermicomposting might also reduce the Zn concentration in the casts, as final products. He et al. (2018) reported that vermicomposting of sewage sludge reduces the heavy metals concentration. Earthworm is known to accumulate heavy metals, so it is expected that Zn concentration might reduce after vermicomposting process. The feasibility of vermicomposting to reduce heavy metals concentration in sludge would be an interesting future research study. Other than vermicomposting, conventional composting process also an option to reduce the heavy metal concentration in the sludge. During the composting process, the sludge will be mixed with other substrates before composting which will reduce its metal concentration.

Compared with the treated wastewater, the Zn and Cu concentration is far less than the limit value. This is because metals are accumulated in the waste activated sludge or solid fraction of wastewater. Treated wastewater effluent (MBRE) utilization for irrigation in the agricultural land would be an option for recycling.

2.4.3. Effect of waste activated sludge and treated swine wastewater on radish and wheat relative seed germination (RSG)

Table 2.3 shows the relative seed germination value of radish and wheat seed grown on WAS and MBRE at different concentration. As WAS and MBRE concentration increase, the relative germinated seed of radish was reduced. In the case of MBRE, RSG were not differed significantly among different concentration of both radish and wheat. This means that MBRE is not affecting the number of germinated seed of both radish and wheat seed. In addition, at each concentration, there is also no significant difference of RSG between radish and wheat in MBRE treatment. Therefore, in term of the number of seed germinated, wheat and radish have the same response towards MBRE.

For WAS treatment, the RSG of radish and wheat was varied among different concentration. The highest RSG for radish was at 10% concentration meanwhile the highest RSG for wheat was at 20% concentration. At low concentration (5 – 20%), the RSG of radish and wheat are not significantly different. However, in general, when the concentration of WAS is 30% and above, the RSG of radish and wheat were significantly different ($p<0.05$). There is an exception at 40% concentration where RSG of radish and wheat were not differed significantly. Based on these results, it can be inferred that wheat and radish have different response towards WAS at high concentration in terms of the number of germinated seed. WAS has higher EC compared with MBRE (Table 2.1) which related with higher salinity. At higher concentration, nutrients and minerals are more concentrated therefore may affect the seed germination process. According to Indra and Mycin (2009), the concentration of salt inhibits seed germination because of osmotic difference causing less absorption of water. Salinity reduced seed germination and inhibit the initiation of germination and seedling establishment (Almansouri et al., 2001). It is also reported that there is an absence of barley seed germination grown on olive mill wastewater with EC of 7.60 mS/cm (Rusan et al., 2015). In general, the results show MBRE has lower seed germination inhibition compared with WAS.

2.4.4. Effect of waste activated sludge and treated swine wastewater on radish and wheat relative root elongation (RRE)

Table 2.4 shows the relative root elongation value of radish and wheat grown on WAS and MBRE at different concentration. The highest root elongation is wheat in 10% concentration of WAS. Dilution of WAS into 10% concentration result in dilution of nutrients which is tolerable for wheat and even enhance the root elongation.

At each concentration, there is significant difference ($p<0.05$) of RRE between radish and wheat in WAS treatment. Based on the result, radish root elongation was significantly lower than wheat root elongation in WAS and MBRE treatment. This indicates that radish exhibit difference response compared with wheat towards WAS at every concentration and starting at 20% concentration of MBRE treatment. Radish might have more sensitivity towards MBRE and WAS. Between WAS and MBRE treatment on radish, there is significant difference on RRE ($p<0.05$) starting at 5% concentration. This means WAS really affects the root elongation on radish seed compared with MBRE. WAS contains more salts, nutrients, and organic matter compared with MBRE which might affect the root elongation of radish. In addition, Zn concentration on WAS which is 1,589 mg/Kg DM, result in phytotoxicity on radish. Study on rhizotoxicity of Zn on wheat and radish shows that radish has higher sensitivity toward Zn than wheat (Pedler et al., 2004).

As WAS and MBRE concentration increase, the relative root elongation

(RRE) of radish was reduced. Meanwhile, for wheat, increase in the concentration of WAS and MBRE results in >100% RRE value up to 40 and 50% concentration, respectively. REE value higher than 100% indicating that root elongation is enhanced compared with control. The enhancement of root elongation is likely due to nutrient availability. Nutrient composition might influence the growth and development of roots. It is known that nitrogen, phosphorus, iron, and sulfur are nutrients that influence the root development. Among the nutrients, increase in P supply known to induce primary root elongation in *Arabidopsis* (Lopez-Bucio et al., 2003). In general, wheat grown on WAS have higher RRE value than grown on MBRE. WAS contains more total phosphorus compared with MBRE (Table 2.1). Therefore, higher P concentration on WAS likely to promote root elongation in WAS treatment of wheat. There is no enhancement of radish root elongation in WAS treatment. It is believed the reason is due to toxicity of other substances to radish which inhibits the root growth at the early stage so that higher phosphorus concentration not affecting the root elongation.

Table 2.3. Relative Seed Germination (RSG) (%) of radish and wheat seed using waste activated sludge (WAS) and membrane bio-reactor effluent (MBRE)

		Relative Seed Germination (RSG) (%)						
Concentration		5	10	20	30	40	50	100
Radish	**WAS**	92.9 ± 16.4^{ab,A}	100.0 ± 6.2^{a,A}	89.3 ± 22.3^{ab,A}	50.0 ± 6.2^{bc,C}	39.3 ± 24.8^{cd,B}	39.3 ± 6.2^{cd,B}	7.2 ± 12.4^{d,B}
	MBRE	100 ± 11.1^{a,A}	100 ± 11.1^{a,A}	96.3 ± 16.9^{a,A}	92.6 ± 6.4^{a,A}	92.6 ± 6.42^{a,A}	96.3 ± 6.4^{a,A}	77.8 ± 19.2^{a,A}
Wheat	**WAS**	83.3 ± 11.5^{ab,A}	83.3 ± 11.5^{ab,A}	96.7 ± 5.8^{a,A}	70.0 ± 10^{ab,B}	66.7 ± 15.3^{b,AB}	80.0 ± 10^{ab,A}	66.7 ± 5.8^{b,A}
	MBRE	96.1 ± 6.6^{a,A}	88.4± 6.6^{a,A}	96.1 ± 6.6^{a,A}	99.9 ± 6.6^{a,A}	92.3 ± 11.5^{a,A}	92.3 ± 11.5^{a,A}	84.6± 6.6^{a,A}

WAS: Waste activated sludge; MBRE: membrane bio-reactor effluent

Value represented as means ± standard deviation (SD) of triplicates

^{a,b} Means within a row followed by different lowercase letter differ significantly ($p<0.05$)

^{A,B} Means within a column followed by different uppercase letter differ significantly ($p<0.05$)

Table 2.4. Relative Root Elongation (RRE) (%) of radish and wheat seed using waste activated sludge (WAS) and membrane bio-reactor effluent (MBRE)

		Relative Root Elongation (RRE) (%)						
Concentration		5	10	20	30	40	50	100
Radish	**WAS**	53.6 ± 6.1^{a,B}	45.6 ± 8.7^{a,B}	19.9 ± 2.1^{b,C}	15.3 ± 1.5^{b,C}	13.6 ± 1.9^{b,D}	19.1 ± 6.4^{b,B}	5.4 ± 9.3^{b,B}
	MBRE	98.2 ± 17.4^{a,A}	99.3 ± 1.0^{a,A}	84.7 ± 12.6^{a,B}	73.9 ± 7.6^{a,B}	44.6 ± 5.4^{b,C}	30.4 ± 10.1^{bc,B}	14.1 ± 1.8^{c,B}
Wheat	**WAS**	130.1 ± 9.1^{ab,A}	150.5 ± 32.9^{a,A}	138.9 ± 8.1^{a,A}	144.4 ± 25.2^{a,A}	149.6 ± 12.5^{a,A}	83.4 ± 8.5^{bc,A}	46.5 ± 10.0^{c,A}
	MBRE	113.3 ± 13.9^{ab,A}	134.9 ± 17.1^{ab,A}	141.8 ± 6.7^{a,A}	140.9 ± 14.3^{a,A}	125.4 ± 10.3^{ab,B}	103.6 ± 15.8^{b,A}	40.8 ± 4.9^{c,A}

WAS: Waste activated sludge; MBRE: membrane bio-reactor effluent

Value represented as means ± standard deviation (SD) of triplicates

^{a,b} Means within a row followed by different lowercase letter differ significantly ($p<0.05$)

^{A,B} Means within a column followed by different uppercase letter differ significantly ($p<0.05$)

2.4.5. Effect of waste activated sludge and treated swine wastewater on radish and wheat germination index (GI)

Table 2.5 shows the germination index (GI) value of seed grown on WAS and MBRE at different concentration. Germination index value >80% considered to be non-phytotoxic while <50% is considered to be highly toxic and not suitable for agricultural purposes (Ravindran et al., 2016). The germination index of waste activated sludge on radish seed at 5% concentration is 49.7% which indicating phytotoxicity. However, for wheat seed, the phytotoxic effect is seen at 100% concentration. As for MBRE, the phytotoxicity is observed at concentration 40% for radish seed and 100 % for wheat seed.

At each concentration, there is significant difference ($p<0.05$) of GI between radish and wheat in WAS treatment and starting at 20% in MBRE treatment. This indicates that radish exhibit different response compared with wheat towards WAS at every concentration and at higher concentration of MBRE. Radish might have more sensitivity towards MBRE and WAS compared to wheat. Between WAS and MBRE treatment on radish, there is significant difference on GI ($p<0.05$) starting at 5% concentration. This means WAS significantly reduced the germination index of radish compared with MBRE. Different type of seed might have different sensitivity towards pollutants result in alteration of seed germination and root elongation thus affecting overall germination index value.

Table 2.5. Germination index (GI) (%) of radish and wheat seed using waste activated sludge (WAS) and membrane bio-reactor effluent (MBRE)

		Germination Index (%)						
Concentration		5	10	20	30	40	50	100
Radish	**WAS**	$49.7 \pm 9.8^{a,B}$	$46 \pm 11.6^{a,B}$	$17.5 \pm 3.7^{b,C}$	$7.7 \pm 1.6^{b,D}$	$5.7 \pm 3.8^{b,C}$	$7.3 \pm 1.6^{b,D}$	$1.2 \pm 1.9^{b,C}$
	MBRE	$97.1 \pm 9.6^{a,A}$	$99.5 \pm 11.1^{a,A}$	$80.5 \pm 11^{ab,B}$	$68.4 \pm 8.4^{b,C}$	$41.1 \pm 2.8^{c,B}$	$29.7 \pm 11.3^{cd,C}$	$11 \pm 3.4^{d,B}$
Wheat	**WAS**	$108.2 \pm 10.3^{ab,A}$	$122.9 \pm 9.2^{ab,A}$	$134 \pm 8.9^{a,A}$	$100.2 \pm 14.2^{b,B}$	$98.5 \pm 15.2^{b,A}$	$63.8 \pm 8.8^{c,B}$	$30.8 \pm 3.8^{d,A}$
	MBRE	$109 \pm 15.9^{cd,A}$	$118.6 \pm 5.5^{abc,A}$	$136 \pm 3.9^{ab,A}$	$140.2 \pm 6.9^{a,A}$	$115.2 \pm 11.4^{bcd,A}$	$94.4 \pm 3.1^{d,A}$	$34.4 \pm 2.7^{e,A}$

WAS: Waste activated sludge; MBRE: membrane bio-reactor effluent

Value represented as means ± standard deviation (SD) of triplicates

^{a,b} Means within a row followed by different lowercase letter differ significantly ($p<0.05$)

^{A,B} Means within a column followed by different uppercase letter differ significantly ($p<0.05$)

Figure 2.2 and 2.3 shows the trends in concentration of waste activated sludge and treated wastewater on the germination index of radish and wheat seed. With increasing concentration, the reduction in germination index was observed in radish seed.

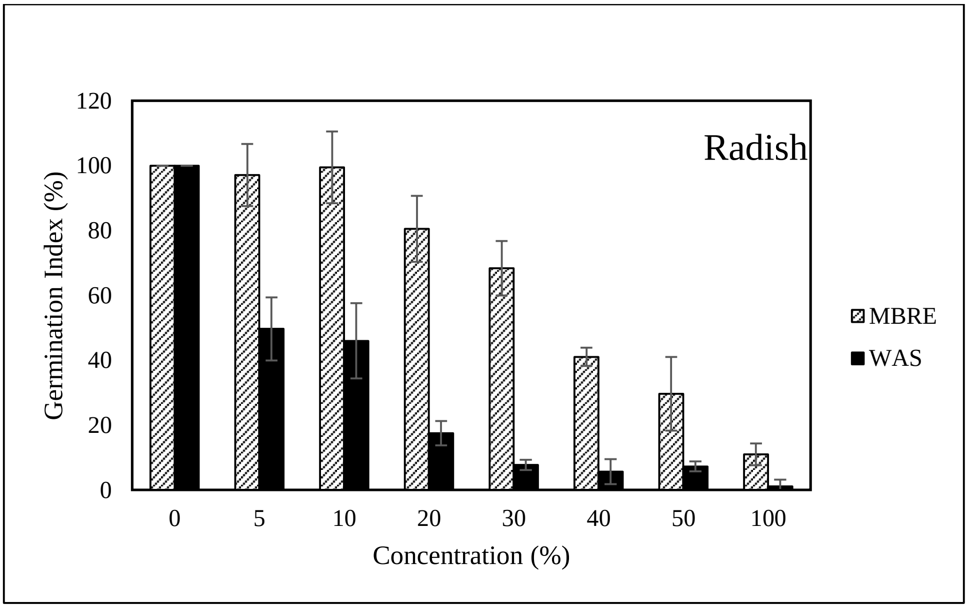

Figure 2.2. Germination index of radish seed using membrane bio-reactor effluent (MBRE) and waste activated sludge (WAS) at different concentration. Error bar represent standard deviation of n =3.

The waste activated sludge at diluted concentration enhance the germination index of wheat seed. However, study by Wollan et al. (1978) reported that fresh sludge affects seed germination which results from high organic matter content. Fresh sludge is untreated since it is the product of primary treatment. Therefore, the organic matter content of fresh sludge is usually high. Meanwhile, waste activated sludge are the products of secondary treatment (such as aerobic wastewater treatment). Some nutrients are converted into available form for plants such as nitrate which is beneficial for plant growth. The organic matter in waste activated sludge is mainly from microbial biomass. It is possible that different type of organic compounds may have different effect on seed germination.

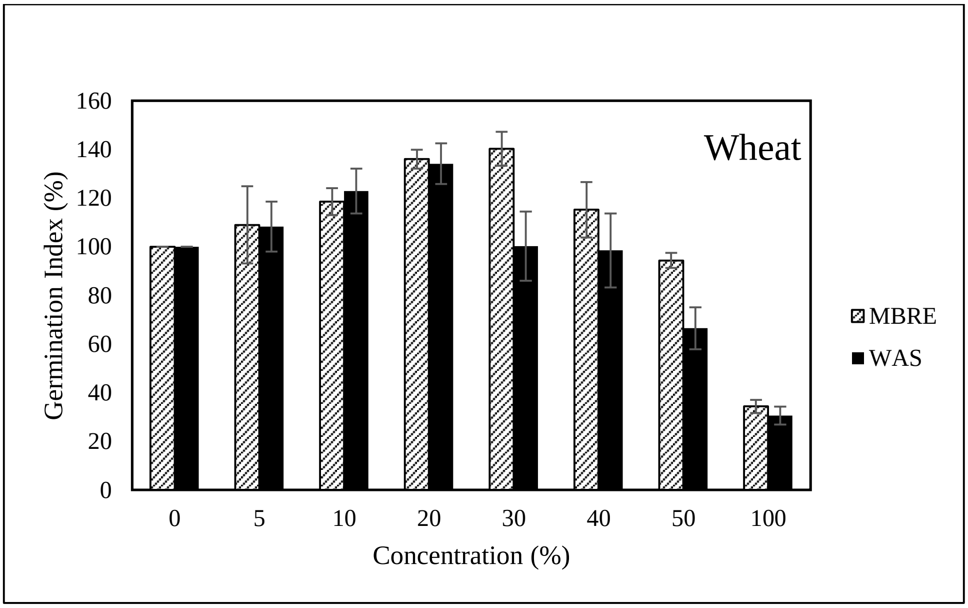

Figure 2.3. Germination index of wheat seed using membrane bio-reactor effluent (MBRE) and waste activated sludge (WAS) at different concentration. Error bar represent standard deviation of n =3.

Based on the graph depicted in Figure 2.3, the increase germination index of treated wastewater is above 100% until it 30% dilution. The increase in germination index indicating that there is root elongation enhancement and number of seed germinated of wheat seed on MBRE. The increase of germination index might be due to increase of nutrient contents which are beneficial for plant growth such as nitrate and ammonium ion. Appreciable amount of nitrate present in the wastewater might stimulate protein production or other organic molecules which is required for plant growth (Yousaf et al., 2010). The same phenomenon also demonstrated on waste activated sludge samples until 20% concentration.

Overall, the response of radish and wheat seed are different towards both WAS and MBRE. Ravindran et al. (2016) reported that the individual crops reacted differently following irrigation with treated and untreated domestic wastewater effluent in which lettuce had the highest sensitivity towards effluent compare to onion, tomato, and carrot.

In the end, the seed germination is a complex process that involves internal and external stimuli (Joosen et al., 2013). The interaction between internal and external stimuli might affect the germination process. Seed germination is a fundamental process prior to the plant growth, therefore, the success of germination is very important. Nutrients affect the germination and root elongation process. The nutrients are external stimuli that will trigger the cellular modification inside the seed to grow and developed further. Based on the result of this study, the nutrient composition and concentration might affect the seed germination and root elongation of radish and wheat.

2.5. Conclusion

Sludge application on land and wastewater utilization for land application is a common practice in waste management. Proper application of treated wastewater and sludge might improve the soil properties and benefit in plant growth. Waste activated sludge and treated wastewater contain nutrients beneficial for plant growth such as nitrogen, phosphorus, potassium, and micronutrients. However, the application of waste activated sludge also poses environmental risks, especially in relation with its salinity indicated by high EC. Waste activated sludge and treated swine wastewater effluent have high EC of 4.95 and 2.4 mS/cm. Higher EC might also affect the seed germination. In addition, high Zn content of 1,589 mg/kg dry matter which is above the permitted limit also observed. The application rate and method should be considered for land application purposes of waste activated sludge (WAS). It was also observed that MBRE is suitable for irrigation purpose since it had less solid content and a considerable amount of nutrients that might have beneficial effect on the plant. In addition, the GI of wheat and radish are higher in MBRE treatment indicating less germination and root elongation inhibition on the plant. However, the dilution of MBRE prior to utilization is important since at 40% concentration it reduces the GI value of radish greatly. At each concentration there is significant difference ($p<0.05$) of GI between radish and wheat in WAS treatment and starting at 20% in MBRE treatment. This indicates that radish exhibit different response compared with wheat towards WAS at every concentration and at higher concentration of MBRE. The germination assay shows that waste activated sludge is phytotoxic start at 5% concentration for radish meanwhile for wheat, the phytotoxicity of waste activated sludge (WAS) was observed at 100% concentration. Treated swine wastewater effluent (MBRE) phytotoxicity was observed at concentration 40% for radish seed and 100 % for wheat seed.

References

Almansouri, M., Kinet, J. M., & Lutts, S. (2001). Effect of salt and osmotic stresses on germination in durum wheat (Triticum durum Desf.). *Plant and soil*, *231*(2), 243-254.

Alvarenga, P., Mourinha, C., Farto, M., Santos, T., Palma, P., Sengo, J., . & Cunha-Queda, C. (2015). Sewage sludge, compost and other representative organic wastes as agricultural soil amendments: Benefits versus limiting factors. *Waste management*, *40*, 44-52.

Apha, A. (2005). WEF, 2005. Standard methods for the examination of water and wastewater, 21, 258-259.

Burkholder, J., B. Libra, P. Weyer, S. Heathcote, D.Kolpin, P.S. Thorne, M. Wichman. 2007. Impacts of waste from concentrated animal feeding operations on water quality. *Environ Health Perspect* 115: 308-312.

Cho, W. M., Ravindran, B., Kim, J. K., Jeong, K. H., Lee, D. J., & Choi, D. Y. (2017). Nutrient status and phytotoxicity analysis of goat manure discharged from farms in South Korea. *Environmental technology*, *38*(9), 1191-1199.

Clarke, B. O., & Smith, S. R. (2011). Review of 'emerging'organic contaminants in biosolids and assessment of international research priorities for the agricultural use of biosolids. *Environment international*, *37*(1), 226-247.

Gao, M., Liang, F., Yu, A., Li, B., & Yang, L. (2010). Evaluation of stability and maturity during forced-aeration composting of chicken manure and sawdust at different C/N ratios. *Chemosphere*, *78*(5), 614-619.

He, X., Zhang, Y., Shen, M., Zeng, G., Zhou, M., & Li, M. (2016). Effect of vermicomposting on concentration and speciation of heavy metals in sewage sludge with additive materials. *Bioresource technology*, *218*, 867-873.

Indra, P., & Mycin, T. R. (2009). Germination changes of varieties of Vigna mungo L. under tannery effluent stress. *Recent Research in Science and Technology*, *1*(5).

Joosen, R. V., Arends, D., Li, Y., Willems, L. A., Keurentjes, J. J., Ligterink, W., ... & Hilhorst, H. W. (2013). Identifying genotype-by-environment interactions in the metabolism of germinating Arabidopsis seeds using generalized genetical genomics. *Plant physiology*, pp-113.

Kiziloglu, F. M., Turan, M., Sahin, U., Kuslu, Y., & Dursun, A. (2008). Effects of untreated and treated wastewater irrigation on some chemical properties of cauliflower (Brassica olerecea L. var. botrytis) and red cabbage (Brassica olerecea L. var. rubra) grown on calcareous soil in Turkey. *Agricultural water management*, *95*(6), 716-724.

Kwon, Y. T., Lee, C. W., & Yun, J. H. (2009). Development of vermicast from sludge and powdered oyster shell. *Journal of Cleaner Production*, *17*(7), 708-711.

Lim, S. J., & Kim, T. H. (2015). Removal of organic matter and nitrogen in swine wastewater using an integrated ion exchange and bioelectrochemical system. *Bioresource technology*, *189*, 107-112.

López-Bucio, J., Cruz-Ramırez, A., & Herrera-Estrella, L. (2003). The role of nutrient availability in regulating root architecture. *Current opinion in plant biology*, *6*(3), 280-287.

Ministry of Environment (MOE). 2017. Livestock excreta management http://eng.me.go.kr/eng/web/index.do?menuId=277 access on 18th September 2018. 10.59 pm.

Pedler, J. F., Kinraide, T. B., & Parker, D. R. (2004). Zinc rhizotoxicity in wheat and radish is alleviated by micromolar levels of magnesium and potassium in solution culture. *Plant and Soil*, *259*(1-2), 191-199.

Priac, A., Badot, P. M., & Crini, G. (2017). Treated wastewater phytotoxicity assessment using Lactuca sativa: focus on germination and root elongation test parameters. *Comptes rendus biologies*, *340*(3), 188-194.

Roig, N., Sierra, J., Nadal, M., Martí, E., Navalón-Madrigal, P., Schuhmacher, M., & Domingo, J. L. (2012). Relationship between pollutant content and ecotoxicity of sewage sludges from Spanish wastewater treatment plants. *Science of the Total Environment*, *425*, 99-109.

Rusan, M. J., Albalasmeh, A. A., Zuraiqi, S., & Bashabsheh, M. (2015). Evaluation of phytotoxicity effect of olive mill wastewater treated by different technologies on seed germination of barley (Hordeum vulgare L.). *Environmental Science and Pollution Research*, *22*(12), 9127-9135.

Singh, R. P., & Agrawal, M. (2010). Biochemical and physiological responses of rice (Oryza sativa L.) grown on different sewage sludge amendments rates. *Bulletin of environmental contamination and toxicology*, *84*(5), 606-612.

Suresh, A., & Choi, H. L. (2011). Estimation of nutrients and organic matter in

Korean swine slurry using multiple regression analysis of physical and chemical properties. *Bioresource technology*, *102*(19), 8848-8859.

Tchobanoglous, G., F.L. Burton, H.D. Stensel. 2005. *Wastewater engineering treatment and reuse*. New York: Mc. Graw-Hill.

Yousaf I, Ali SM, Yasmin A.2010. Germination and early growth response of Glycine max varieties in textile and paper industry effluents. *Pak J Bot.* 42:3857–3863.

CHAPTER 3. Bio-methane potential of waste activated sludge derived from swine wastewater treatment

This chapter will be published in SCI(E) journal as partial fulfillment of Agricultural Biotechnology's Ph.D. program for Sartika Indah Amalia Sudiarto

Abstract

Anaerobic digestion is one of sustainable option for waste activated sludge utilization. In this study, the batch bio-methane potential assay was conducted. The substrates are waste activated sludge (WAS) from swine wastewater treatment system, swine slurry (SS), water lily (WL), and lotus (LT) shoot biomass. Co-digestion of waste activated sludge with swine slurry or water lily or lotus shoot biomass are also investigated. The bio-methane potential value of waste activated sludge is ranged from 255.2 – 468.9 NmL CH_4 /g VS-added. Co-digestion of waste activated sludge with swine slurry, water lily, or lotus shoot biomass produce synergistic effect with $\alpha > 1$ indicating that co-digestion improve the methane potential yield. The maximum cumulative methane yield of substrate co-digestion is more than 500 NmL CH_4 /g VS-added in all substrates. The increase of methane yield of co-digestion of WAS with SS, WL, and LT are 14.89, 10.97, and 16.89% respectively. Swine slurry, water lily, and lotus biomass are potential co-digestion substrate for waste activated sludge anaerobic digestion.

Keywords: *Batch anaerobic digestion, waste activated sludge, swine slurry, plant biomass, co-digestion*

3.1. Introduction

Activated sludge process of wastewater is one of major technology for wastewater treatment. Activated sludge process is able to treat various type of wastewater from sewage, municipal, industrial, and agricultural wastewater such as livestock wastewater. However, besides its benefit, the end products of activated sludge process are the solid waste which known as bio-solid or waste activated sludge. The disposal of solid waste generated from this process cause problem especially, the policies regarding solid waste disposal are become stricter. Therefore, it is essential to find a way to manage the solid generated from activated sludge process.

Anaerobic digestion technology is an alternative to manage waste activated sludge. Anaerobic digestion process produces less sludge than activated sludge process happens in aerobic condition. In addition, through anaerobic digestion renewable energy in form of methane gas can be obtained. The digestate obtained from anaerobic digestion process also consider as valuable resources for agriculture fertilization.

With the increase in livestock production, South Korea also facing environmental problems because of livestock waste disposal. The major livestock commodity in South Korea is swine. Most of the swine production facilities are slatted pit housing system. The produced waste is in the form of slurry wastewater. The South Korean governmental policies are focused on the prevention of environmental pollution from livestock excreta. However, according to Ministry of Environment (MOE) (2017), the waste management point of view has been changed towards the conversion of livestock waste into renewable energy source. Anaerobic digestion technology is in accordance with the government program. The waste activated sludge derived from the livestock wastewater treatment can be utilized as feedstock for bioenergy production.

Several ways to treat sludge including landfilling, combustion, and composting. Anaerobic digestion is one of sustainable option since the process degrades the sludge in addition produced energy which can be converted into heat or electricity. It has been known that sewage sludge management through anaerobic digestion is one of sustainable option for waste sludge treatment (Tyagi and Lo, 2013; Cao and Pawlowski, 2012). The treatment also expected to reduce the volume, improve its character and reduce associated environmental risks (Appels et al., 2008). Anaerobic digestion of waste sludge potentially reduces its volume since it converted the organic matter into biogas (60 – 70% methane), remove pathogens, and inhibit the odor emission (Appels et al., 2008) if compared with direct land application. In South Korea, it is reported that 20 biogas plants out of total 49 biogas plants are using sewage sludge as its feedstock (Kim et al., 2012).

Anaerobic digestion process converts biodegradable organic material without oxygen presence into smaller molecules in addition to biogas production. The anaerobic process thus should be conducted in closed reactors. This provides benefit in reducing the odor contamination into the environment since the process happens in closed system. Waste sludge derived from swine wastewater treatment contain organic matter and odor substances that potentially will release during the breakdown.

Waste activated sludge contains less organic matter compared with raw swine wastewater which may reduce its potential to produce energy. Co-digestion is one method to increase the biogas potential. Livestock waste is commonly utilized to enhance biogas production. Co-digestion of pig manure with sewage sludge increase the buffering capacity, improve nutrient balance and produce synergism (Zhang et al., 2014). The use of livestock waste as co-substrate might be an option to enhance biogas production. The utilization of livestock waste also will help to reduce the environmental impact of livestock production. Co-digestion with substrate rich in organic matter will then increase its bio-methane potential. Zhang et al. (2014) reported that co-digestion of dewatered sewage sludge with pig manure increase its methane yield by 82.4%.

On the other hand, in wastewater treatment process, especially dealing with high nutrient content wastewater such as swine wastewater, further nutrient removal of effluent is needed to reach the discharge limit. Nutrient removal by plants is one of tertiary treatments of wastewater effluent. Aquatic plants have been widely used for nutrient removal from wastewaters. Floating and emergent plants have been studied for nutrient and heavy metal removal from different types of wastewater (Muradov et al., 2014; Seo et al., 2010; Tel-Or and Forni, 2011). The study on nutrient removal from treated swine wastewater by aquatic plants has been done in Suwon, Seoul National University experimental farms to remove excess nitrogen from wastewater effluents utilizing floating aquatic plants (Sudiarto et al., 2019) and emergent plants, *Miscanthus sacchariflorus, Phragmites australis, Nymphaeae* sp., and *Nelumbo nucifera*. The bio-methane potential of *Miscanthus* and *Phragmites* grown on treated wastewater have been reported by Sudiarto et al. (2015). *Miscanthus sacchariflorus* and *Phragmites australis* are known as bioenergy crops meanwhile *Nymphaeae* sp. (water lily) and *Nelumbo nucifera* (Lotus) are mostly known as ornamental plants. Bio-methane potential from water lily and lotus is not widely studied. However, water lily and lotus plant biomass might also have potential as anaerobic digestion substrate or co-substrate.

The sustainability of phytoremediation also determined by how is the biomass utilized after it is used in the nutrient removal system. Most of the study about phytoremediation mainly focus on nutrient removal itself with the biomass production without more observation on the biomass potential value. The integration of nutrient removal study with biomass potential value will give an idea of the added value of the plant biomass that can be obtained from the nutrient removal system. Co-digestion of waste activated sludge and macroalgae has been reported by Costa et al. (2012). The increase of methane yield by co-digestion is 26%. Arias et al. (2018) studied on co-digestion of microalgae with waste activated sludge derived from municipal wastewater treatment plant. The combination of waste activated sludge and biomass derived from phytoremediation might be one of the option of biomass utilization as well as sludge management. It is also expected that co-digestion of waste activated sludge with plant biomass might improve the methane production. In this study, shoot biomass of *Nymphaeae* sp. (Water lily), and *Nelumbo nucifera* (Lotus) will be used as substrate for co-digestion with waste activated sludge.

3.2. Objectives

1. Determine the bio-methane potential of waste activated sludge, swine slurry, water lily, and lotus shoot biomass.
2. Determine the bio-methane potential of co-digestion of waste activated sludge and swine slurry or plant biomass.

3.3. Materials and Method

3.3.1. Inoculum and Substrate

Inoculum was obtained from mesophilic anaerobic digestion reactor treating swine slurry. The inoculum was maintained in 200 mL serum bottle at 35°C. Prior to use in the experiment, the inoculum was degassed. Substrates for bio-methane potential were swine slurry, waste activated sludge, dried water lily, and lotus shoot biomass. The swine slurry was obtained from pit type experimental housing system occupied with 10 growing pigs. The water lily and lotus plants were grown on treated swine wastewater effluent from Suwon experimental farm, Seoul National University. The biomass consists of dried leaves and stalks of water lily and lotus plants. The dried biomass then grinds and passed through 1 mm mesh. Waste activated sludge was obtained from small-scale swine wastewater treatment process treating about 90 L swine slurry per days. The treatment process consists of anoxic and oxic reactors, sedimentation tank, and filtration tank. The waste activated sludge was obtained from the sedimentation tank.

3.3.2. Characteristics of substrates

a. Total solid, volatile solid, and fixed solid

Total solid, volatile solid and fixed solid of waste and activated sludge and swine slurry were determined according to APHA standard method 2450 (APHA, 2005). About 10 mL well mixed samples of waste activated sludge and swine slurry were weighed and dried in an oven at 103-105 ˚C, then the total solid was calculated and expressed as mg/L fresh weight or % weight. After total solid measurement, the residue was ignited in muffle furnace at a temperature of 550 ˚C. The volatile and fixed solid were calculated and expressed as mg/L fresh weight or % dried weight.

b. Plant biomass characteristics

For plant biomass analysis, air-dried samples were used. The organic matter and ash content are determined based on loss on ignition method. Approximately 0.1 – 0.2 g of dried samples are ignited in the muffle furnace at 550°C for 2 hours. The residue after ignition represents the ash content and the combusted material represent the organic matter of the sample. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) analyses were performed by a filter bag method utilizing an ANKOM F57 filter. Approximately 0.5 g sample was placed inside the bag, sealed, and then refluxed with ADF or NDF reagents at 200°C for 1.5 hours. After that, the filter bag was rinsed with warm water until the rinsed water becomes clear. The bags were then soaked and washed with acetone, dried at 105°C for 2 hours, and the weights were measured. After ADF analysis, the filter bag was kept submerged in beaker glass filled with 72% H_2SO_4 for 3 hours, in which every 30 minutes the bags were agitated. Afterward, the bags were washed with warm distilled water until pH is neutral, then washed with acetone and dried at 105°C. The NDF, ADF, and ADL were determined by weight difference and converted into dry matter basis.

c. Ultimate analysis of substrates

The ultimate analysis measuring carbon, hydrogen, oxygen, nitrogen, and sulfur was determined by Prior to ultimate (elemental) analysis, samples were dried and sieved through 1 mm mesh. The carbon, hydrogen, nitrogen, and sulfur content of pretreated samples were analyzed with Elemental Analyzer (Flash EA 1112, Thermo Fisher Scientific, Germany) via combustion at 1014°C. The oxygen content of the samples was analyzed with another instrument (Flash 2000 Elemental Analyzer, Thermo Fisher Scientific, Germany).

3.3.3. Anaerobic media

The anaerobic medium was made according to Shelton and Tiedje (1984) and Angelidaki et al (2009). The anaerobic media consist of phosphate buffer solution, mineral salts, trace metal solution, and sodium bicarbonate. Each media was made separately as a stock solution and were diluted and mixed prior to the experiment.

3.3.4. Batch bio-methane potential (BMP) assay of waste activated sludge and co-digestion with swine slurry and plant biomass

The experiment was divided into *Experiment I* and *Experiment II*. The *Experiment I* was conducted on single substrate while *Experiment II* evaluated the co-digestion of substrates on bio-methane potential. Waste activated sludge samples of the plants were put into 250 ml serum bottles. The weight was adjusted so that each serum bottle contained 0.5 g or 0.25 g of volatile solids of swine slurry, waste activated sludge, plant biomass, or mixture of waste activated sludge with swine slurry or plant biomass. The treatment, substrate mixture, and Substrate/Inoculum (S/I) ratio are presented in Table 3.1. Each treatment was triplicated. An anaerobic test media containing phosphate buffer, macronutrients, and micronutrients was added, and 21 ml inoculum of anaerobic microorganisms was added to bring the total volume to 200 ml. Then 50 mL sample from each serum bottles was taken for further analysis. Blanks were prepared using the anaerobic medium and inoculum to correct biogas production. The mixture was then purged with N_2/CO_2 gas to remove oxygen for 5 minutes. The serum bottle was closed with a rubber cap and then sealed with aluminum crimps. All bottles were put into the incubator, which maintained a temperature of 35°C.

Table 3.1. Treatments of batch bio-methane potential (BMP) assay in this study

No.	Substrate	VS added	S/I ratio
Experiment I			
1	Waste activated sludge (WAS)	0.5, 0.25	1 : 1, 1:2
2	Swine slurry (SS)	0.5, 0.25	1 : 1, 1:2
3	Water lily shoot biomass (WL)	0.5, 0.25	1 : 1, 1:2
4	Lotus shoot biomass (LT)	0.5, 0.25	1 : 1, 1:2
Experiment II			
5	SS + WAS (SSAS 1) (1 : 1)	0.25	1 : 2
6	SS + WAS (SSAS 2) (1 : 2)	0.25	1 : 2
7	LT + WAS (LTAS) (1 : 2)	0.25	1 : 2
8	WL + AS (WLAS) (1 : 2)	0.25	1 : 2

VS: Volatile solid; S/I : Substrate/Inoculum

3.3.5. Theoretical methane potential based on ultimate analysis

Theoretical methane potential is used to estimate methane production of certain substrate. The theoretical methane potential is expressed as volume of methane at standard temperature and pressure. To obtain the potential methane production, the C, H, O, N, S composition of substrate of interest should be known. From the analysis results, the specific chemical formula of the analyzed substrate can be obtained. The empirical chemical formula ($C_aH_bO_cN_dS_e$) was estimated by using the results obtained from the elemental analysis. Methane production in anaerobic condition from organic matter with its respective molecular formula was calculated based on formula developed and elaborated by Symon and Buswell (1933) and Boyle (1976) according to Moukazis et al (2018) as follow:

$$C_aH_bO_cN_dS_e + \left(a - \frac{b}{4} - \frac{c}{2} + \frac{3d}{4} + \frac{e}{2}\right) H_2O \rightarrow \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4}\right) CH_4 + \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4} + \frac{3d}{8} + \frac{e}{4}\right) CO_2 + dNH_3 + eH_2S \qquad (5)$$

The theoretical methane potential (TMP) of single substrate at standard temperature and pressure (STP), 273 K and 1 atm, was calculated based on stoichiometric conversion of substrate molecular formula using equation (6).

$$TMP\ (ml\ CH_{4STP}) = 22.4 \times \frac{CH_4\ coeff.}{Mr} \times 1000 \qquad (6)$$

3.3.6. Gas production and composition analysis and measurement

Biogas production was measured with a glass syringe using the pressure displacement method. The gas composition (CO_2, CH_4, and N_2) was analyzed using Gas Chromatography (Agilent) equipped with a column HP-PLOT/Q and a Thermal Conductivity Detector (TCD). The inlet, oven, and detector temperature were 40, 35, and 200°C, respectively. The cumulative methane gas production was calculated by using the following equation:

$$V_{CH_4t} = V_b \times C_{CH_4} \qquad (7)$$

where,

V_{CH_4t} : Volume of methane at time t

V_b : Volume of biogas at time t

C_{CH_4} : Concentration of methane at time t

Meanwhile, the cumulative methane production was calculated by following equation,

$$V_{CH_4cum} = V_{CH_4t_i} + V_{CH_4t_{ii}} + \cdots + V_{CH_4t_x} \qquad (8)$$

where,

V_{CH_4cum} : Cumulative methane production (mL)

The measured cumulative methane production was adjusted to the volume at standard condition at 273 K and 1 atm. The cumulative CH_4 content was then utilized to determine experimental methane yield (EMY) as follow:

$$\text{EMY} = V_{CH_4cum}/g\ VS - added \qquad (9)$$

where:

V_{CH_4cum} : Cumulative CH_4 production, in Nml

EMY : Experimental CH_4 yield, in Nml/g VS added

VS-added : Volatile solid of initial sample, in g

3.3.7. Kinetic model

According to Kafle et al (2013), the first order kinetic model is less fit than Gompertz kinetic model. Gompertz model takes lag phase into the calculation since it is also important parameter in the batch bio-methane potential assay of complex organic substrate. Therefore, the kinetic model in this study is based on the Gompertz equation.

$$G(t) = G_0 \, exp\left\{-exp\left[\frac{R_{max}e}{G_0}(\lambda - 1) + 1\right]\right\} \quad (10)$$

where:

G (t) : Cumulative methane yield at digestion time (t) (mL/g VS added)

G_0 : Maximum methane yield of substrate (mL/g VS added)

$R_{max}{}^{e}$: Maximum methane production rate (mL/g VS-added)

λ : Lag phase (day)

t : Time (day)

e : exp (1) = 2.7183

3.3.8. Biodegradability

The anaerobic biodegradability of anaerobic digestion assay is calculated based on the comparison on theoretical methane potential of the substrate. According to Raposo et al. (2012), the anaerobic biodegradability (D_{deg}) calculated as follows:

$$D_{deg} = \frac{EMY}{TMP} \times 100 \quad (11)$$

where,

EMY = Experimental methane yield, Nml CH_4/g VS-added

TMP = Theoretical methane potential, Nml CH_4/g VS-added

3.3.9. Synergistic effect

The synergistic effect measures the contribution of each substrate in final methane production in the co-digestion experiment. The synergistic effect was calculated based on the equation according to Nielfa et al (2015) (equation 12).

$$\alpha = \frac{Maximum\ methane\ production\ (G_0)}{Calculated\ methane\ production\ (CMP)} \tag{12}$$

$\alpha > 1$: Substrate mixture have synergistic effect.

$\alpha = 1$: Substrate mixture do not have synergistic nor antagonistic effect.

$\alpha < 1$: Substrate mixture have antagonistic effect.

The G_0 is maximum bio-methane potential for each substrate co-digestion mixture while the CMP is the value obtained from the sum of BMP of each sole substrates considering the VS proportion of each substrate contained in the mixture. G_0 is the maximum methane potential calculated based on the Gompertz kinetic model.

3.3.10. Statistical analysis

The analysis of variance (ANOVA) and Tukey test were used to determine the differences among treatments. Confidence interval of 95% was used for data analysis. The statistical analysis was performed by using Excel software 2016.

3.4. Results and Discussions

3.4.1. Substrate characteristics

Table 3.2 shows the characteristics of substrate used in this study. Plant shoot biomass of water lily and lotus contains higher volatile solid per total solid which means both have higher organic matter than waste activated sludge and swine slurry. The total solid of WAS in this study was 6.6% which is higher than secondary municipal sludge which is 3.48 % and lower than primary municipal sludge of 8.6% (Costa et al., 2012). The volatile solid of WAS is 3.75% which is higher than reported volatile solid value of secondary municipal sludge of 2.56% (Costa et al., 2012).

Plant biomass contains more carbon since plant cell wall contains cellulosic material. WAS contains the least carbon than other substrates since it is derived from wastewater treatment process. The swine wastewater treatment process removes carbon from swine wastewater and converts it into carbon dioxide, therefore, the carbon content in end products (WAS) is less.

Interestingly, the hemicellulose content and cellulose content of WAS higher than swine slurry. The determination of cellulose, hemicellulose, and lignin content of the substrates was based on known method of Van Soest. This method usually used to determine the cellulose, hemicellulose, and lignin content of plant materials. In this case, waste activated sludge (WAS) is unlikely have same or higher cellulosic and lignin content with plant materials.

WAS component mostly being microbial biomass which has higher protein content than fiber. However, most of the studies regarding waste activated sludge (WAS) characteristics on cellulosic and lignin material composition is scarce. High cellulose, hemicellulose, and lignin content in WAS might be representing materials which are not really cellulose, hemicellulose, or lignin, therefore, represent cellulosic-like and lignin-like materials.

Table 3.2. Characteristics of substrates used in batch Bio-methane Potential (BMP) assay

Parameters	Waste Activated Sludge	Swine slurry	Water lily	Lotus
Total solid (TS) (% FW)	6.60 ± 0.01	4.12 ± 0.21	11.57 ± 0.84	12.41 ± 1.19
Volatile solid (VS) (% FW)	3.75 ± 0.02	2.79 ± 0.17	10.00 ± 0.86	10.65 ± 1.14
VS/TS	0.57 ± 0.004	0.68 ± 0.01	1.57 ± 0.02	1.76 ± 0.05
C (%DW)	29.43 ± 0.26	37.31 ± 0.33	42.16 ± 1.01	40.12 ± 1.26
H (%DW)	4.26 ± 0.01	5.23 ± 0.01	5.50 ± 0.39	5.67 ± 0.27
O (%DW)	21.43 ± 1.67	23.66 ± 0.69	37.75 ± 1.89	35.48 ± 1.08
N (%DW)	3.81 ± 0.17	4.79 ± 0.13	3.76 ± 0.34	2.94 ± 0.71
S (%DW)	0.79 ± 0.15	1.02± 0.06	0.23 ± 0.05	0.22 ± 0.02
C/N	7.72 ± 0.41	7.78 ± 0.15	11.21 ± 0.69	13.64 ± 1.12
Cellulose (%DW)	13.5 ± 0.24	9.9 ± 0.30	17.4 ± 1.5	27.9 ± 1.5
Hemicellulose (%DW)	36.4 ± 5.09	16.9 ± 1.09	6.0 ± 1.4	9.7 ± 4.6
ADL (%DW)	10.0 ± 0.18	4.2 ± 0.29	1.8 ± 0.5	7.3 ± 1.2

FW : Fresh weight; DW: Dry weight; C: Carbon; H: Hydrogen; O: Oxygen; N: Nitrogen; S: Sulfur; ADL : Acid detergent lignin

Value represented as means ± standard deviation (SD)

Study by Mottet et al. (2010) fractionated WAS solid material derived from municipal wastewater treatment as hemicellulose-like, cellulose-like, and lignin-like fraction as an approach to map the degradability of WAS. The fraction was represented around 37% of total solids. However, in this study, the total fraction of cellulosic-like and lignin-like content from WAS seem to be overestimated. It also has been reported that waste sewage sludge contains more than 20% cellulose content (Honda et al., 2002), however, this is because the sewage sludge is primary sludge and contain fraction of toilet paper.

High cellulose content in plant biomass, lotus, and water lily, might affect the biogas production. To improve the degradability of the lignocellulosic material, pre-treatment is often conducted. For example, alkaline pre-treatment of rice straw and thickened waste activated sludge reduces the lignocellulosic content of the material thus improving the biogas production (Abudi et al., 2016).

3.4.2. Methane production of waste activated sludge, swine slurry, and plant biomass at different S/I ratio

Substrate/Inoculum (S/I) ratio is one of important factor in anaerobic digestion process. Common S/I ratio to be used in batch anaerobic assay is 1 based on volatile solid (VS) (Gunaseelan, 1997). Less S/I ratio in some cases increases the methane production as reported by Chynoweth et al (1993) while using plant biomass with S/I 0.5 VS/VS. In this study, the S/I ratio of single substrate was 1 and 0.5. The experimental cumulative methane yield (EMY) for all single substrate increased with decrease of S/I ratio. There was significance different

($p<0.05$) of EMY between S/I ratio 1 and 0.5 of each substrate. This indicates that S/I ratio affect the methane production.

The experimental methane yield (EMY) obtained from all single substrate shown in Table 3.3. The EMY of waste activated sludge was 259.35 and 460.88 NmL CH_4/g VS-added for S/I 1 and 0.5 respectively. It was the least EMY among other substrates. Based on its characteristics (Table 3.2), waste activated sludge contains the lowest carbon content among the substrates.

In addition, sludge is known to be less biodegradable due to its nature. Majority of organic compounds in the waste activated sludge are in the form of microbial cells. Degradation of such microbial cell is the main problems to further process or utilize waste activated sludge. The structure of cell membranes protects it from osmotic lysis and the cell wall of microbes consist of strands of glycan cross-linked by peptide chain that is hard to be biodegraded. Therefore, prior to treatment or utilization, microbial cells should be broken down to release its contents, which then will be easier to be converted into simple compounds (Weemaes & Verstraete, 1998). Hydrolysis is one of limiting factor in anaerobic digestion process of waste sludge (Appels et al., 2008). Pre-treatment of sludge prior to anaerobic digestion might provide alternative to increase its methane potentials. However, sludge pre-treatment will increase the operational cost which should be taken into account.

Table 3.3. Empirical chemical formula of substrate, TMP based on ultimate analysis, EMY of single substrates and degradability (D_{deg})

Parameters	WAS		SS		WL		LT	
Empirical formula	$C_{10.1}H_{17.5}O_{5.5}N_{1.2}S_{0.1}$		$C_{9.8}H_{16.5}O_{4.7}N_{1.2}S_{0.1}$		$C_{47.1}H_{74.0}O_{32.7}N_{3.7}S_{0.1}$		$C_{51.6}H_{86.0}O_{33.1}N_{3.7}S_{0.1}$	
TMP (NmL CH_4/g VS-added)	494.03 ± 30.00 ^{ab}		529.54 ± 9.02 ^{a}		450.78 ± 8.05 ^{b}		471.04 ± 5.68 ^{ab}	
	WAS		**SS**		**WL**		**LT**	
S/I	**1:1**	**1:2**	**1:1**	**1:2**	**1:1**	**1:2**	**1:1**	**1:2**
EMY (NmL CH_4/g VS-added)	259.35±5.74^{f}	460.88±13.62^{bc}	317.83±10.05^{e}	524.45±5.78^{a}	396.13±7.53^{d}	434.70±18.30^{c}	375.25±11.65^{d}	492.96±3.59^{ab}
D_{deg}	52%	93%	60%	99%	88%	96%	80%	104.6%

WAS: Waste activated sludge; SS: Swine slurry; WL: Water lily; LT: Lotus; VS: Volatile solid TMP : Theoretical Methane Yield ; EMY : Experimental Methane Yield; S/I: Substrate/Inoculum; D_{deg} : Degradability of anaerobic digestion

Value represented means ± standard deviation (SD)

^{a,b} Means within a row followed by different lowercase letter differ significantly ($p<0.05$)

The EMY of swine slurry was 317.83 and 524.45 NmL CH_4/g VS-added for S/I 1 and 0.5 respectively. Swine slurry has been commonly utilized as anaerobic digestion process. Methane yield of 347, 358.7, and 437 mL CH_4/g VS-added has been reported (Bonmati et al., 2001; Zhang et al., 2014; Chae et al., 2008). Compare with the waste activated sludge EMY, swine slurry is 55% higher for S/I 1 and 53% higher in S/I 0.5. The EMY of WAS and SS are different significantly ($p<0.05$) whether at S/I ratio 1 or 2. WAS characteristics might influence the methane production. Even though the volatile solid content of SS is lower than WAS (Table 3.2), it does not result in higher methane production. The volatile solid present in SS is likely more degradable than volatile solid fraction of WAS.

Experimental cumulative methane yield for water lily are 396.13 and 434.70 NmL CH_4/g VS-added for S/I 1 and 0.5 respectively. Meanwhile for lotus are 375.25 and 492.96 NmL CH_4/g VS-added for S/I 1 and 0.5 respectively. The EMY for both plant biomasses only had slight difference. The EMY of water lily and lotus was not significantly different at S/I ratio of 1 ($p>0.05$). However, it is significantly different ($p<0.05$) at S/I ratio of 0.5.

Empirical chemical formula of 4 substrates was shown in Table 3.3. The theoretical methane potential (TMP) of each substrate based on its ultimate analysis are also presented. Swine slurry has the highest TMP among other substrates while water lily has lowest TMP. Swine slurry (SS) has significantly higher ($p<0.05$) TMP compare to water lily. Meanwhile, the TMP of WAS, LT, and SS did not differ significantly.

The degradability of anaerobic digestion increased at S/I ratio of 0.5. The degradability of substrate supposed to be less than 100% since there is energy utilized for microbial growth. However, in this experiment, the degradability of lotus at S/I 1:2 is more than 100%. First possible reason is, the theoretical methane value calculation is underestimated because of using calculation for empirical formula considering sulfur (S) content. Plant biomass contains very less amount of S compared to swine slurry and waste activated sludge so, the calculation based on stoichiometric relationship by Tchobanoglous (1993) according to Chen et al., (2008) would be preferable. Second, there is additional VS degradation which comes not from the substrate itself, but from the inoculum. Study on batch bio-methane potential assay by Yoon et al. (2014) observed the same phenomenon when the S/I ratio is 0.1. Less S/I ratio might have contributed to the unusual degradability value.

Overall, decrease in S/I resulted in higher degradability. It is known that high amount of inoculum affects the rate of biodegradation. The higher the inoculum, the faster the anaerobic digestion process (Raposo et al., 2011).

Table 3.4. Gompertz kinetic model parameter of single substrate and experiment

Kinetic parameter	SS		WAS		LT		WL		SSAS 1	SSAS 2	LTAS	WLAS
	S/I 1:1	S/I 1:2	S/I 1:1	S/I 1:2	S/I 1:1	S/I 1:2	S/I 1:1	S/I 1:2	S/I 1:2			
R_{max} (NmL/g VS added/d)	35.9 ± 10.80^{a}	59.23 ± 12.86^{b,B}	24.71 ± 4.80^{a}	45.56 ± 5.86^{ab,AB}	26.14 ± 0.22^{a}	43.13 ± 1.41^{ab,AB}	27.84 ± 2.03^{a}	36.11 ± 0.91$^{ab.A}$	41.14 ± 4.16^{A}	45.15 ± 2.42^{AB}	41.01 ± 2.24^{A}	43.58 ± 0.69^{AB}
λ (d)	0.11 ± 0.19^{a}	0.92 ± 0.67^{ab,A}	1.84 ± 0.48^{bc}	2.65 ± 0.30^{cd,BC}	3.58 ± 0.61^{d}	3.90 ± 0.27^{d,D}	3.02 ± 0.37^{cd}	3.33 ± 0.23^{d,CD}	2.11 ± 0.42^{B}	2.22 0.16^{BC}	4.15 ± 0.05^{D}	3.69 ± 0.23^{D}
G_0 (Nml CH_4/g VS-added)	293.90 ± 10.41^{b}	495.58 ± 5.35^{e,BC}	241.30 ± 4.63^{a}	441.61 ± 13.94^{d,A}	358.30 ± 5.56^{c}	478.89 ± 6.22^{e,B}	386.15 ± 11.89^{c}	423.61 ± 3.17^{d,A}	507.38 ± 12.37^{BC}	506.69 ± 8.28^{BC}	490.08 ± 19.38^{BC}	516.21 ± 5.13^{C}
EMY (NmL CH_4/ g VS-added)	317.83 ± 10.05^{e}	524.45 ± 5.78^{a,CD}	259.35 ± 5.74^{f}	460.88 ± 13.62^{bc,AB}	375.25 ± 11.65^{d}	492.96 ± 3.59^{ab,BC}	396.13 ± 7.53^{d}	434.70 ± 18.30^{c,A}	532.80 ± 14.36^{CD}	534.36 ± 8.72^{D}	510.90 ± 20.04^{CD}	538.76 ± 6.52^{D}
R^2	0.98	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99
Difference of EMY and G_0 (%)	7.53	5.50	6.96	4.18	4.52	2.85	2.52	2.55	4.77	5.18	4.07	4.19

WAS: Waste activated sludge; SS: Swine slurry; WL: Water lily; LT: Lotus; SSAS 1: Swine slurry- waste activated sludge 1:1; SSAS 2: Swine slurry- waste activated sludge 1:2; WLAS: water lily- waste activated sludge; LTAS: lotus-waste activated sludge;

EMY: Experimental Methane Yield; S/I: Substrate/Inoculum; R_{max}: Maximum methane production rate; λ: lag phase (day); G_0: maximum methane yield (model); EMY: Experimental methane yield

Value represented means ± standard deviation (SD)

[a,b] Means of single substrate within a row followed by different lowercase letter differ significantly ($p<0.05$)

[A,B] Means at S/I ratio 0.5 within a row followed by different uppercase letter differ significantly ($p<0.05$)

3.4.3. Gompertz kinetic model and co-digestion experiment

The Gompertz kinetic model was introduced to predicted maximum methane yield (G_0), maximum methane production rate (R_{max}), and lag phase (λ). The parameters' values of Gompertz model were presented in Table 3.4. To evaluate the fitness of the model, the experimental Figure 3.1 and 3.2 shows plotted experimental and predicted maximum methane potential values of single substrate and co-digestion according to Gompertz model. The R^2 value of 0.94 – 0.99 were obtained. Similar R^2 value range was also obtained on previous study employing Gompertz kinetic model (Zhang et al., 2014; Kaffle, 2013).

The lowest R_{max} value was obtained when waste activated sludge at S/I 1. The improvement of R_{max} was obtained at S/I ratio 0.5 and co-digestion. Lower S/I ratio means more microbes compared to substrate which may be attributed to the acceleration of substrate conversion. The substrate mixing might accelerate the anaerobic digestion process result in increase of methane production rate.

The lag phase represents the time needed for the microorganism to adapt to new substrate. During this period, the hydrolysis of substrate taken place. Longer lag phase period may be attributed to the degradability of the substrate. Swine slurry has the lowest lag phase of 0.11 d at S/I 1:1. According to previous study by Zhang et al. (2014), the lag-phase period of pig manure according to Gompertz kinetic model was 6.9 day meanwhile for dewatered sewage sludge (DSS) was 1.8 day, which is the opposite of the results of this study. In this study, the lag-phase period of WAS was higher than swine slurry. Swine slurry and pig manure might have different characteristics. Swine slurry contains more water makes it more diluted than pig manure. In addition, initial fermentation might also happen since it is easier to be broken down because of more water content result in more simple compounds available in swine slurry. The simple compounds will be ready to be converted as soon as possible makes it has low lag-phase period.

Plant biomass has higher lag-phase period. Waste activated sludge also has considerably higher lag phase period. For substrate that is not easy to degrade, the lag phase period will be longer. The waste activated sludge might have properties that make initial hydrolysis took longer than swine slurry. As described earlier, majority of organic compounds in the waste activated sludge are in the form of microbial cells. Degradation of such microbial cell is the main problems to further process or utilize waste activated sludge. The structure of cell membranes protects it from osmotic lysis and the cell wall of microbes consist of strands of glycan cross-linked by peptide chain that is hard to be biodegraded. In the case of plant biomass, cellulosic material in its cell components might inhibit the initial hydrolysis of the substrate, therefore, increasing lag phase period. In addition, the inoculum used in this study might not adapt to the cellulosic material.

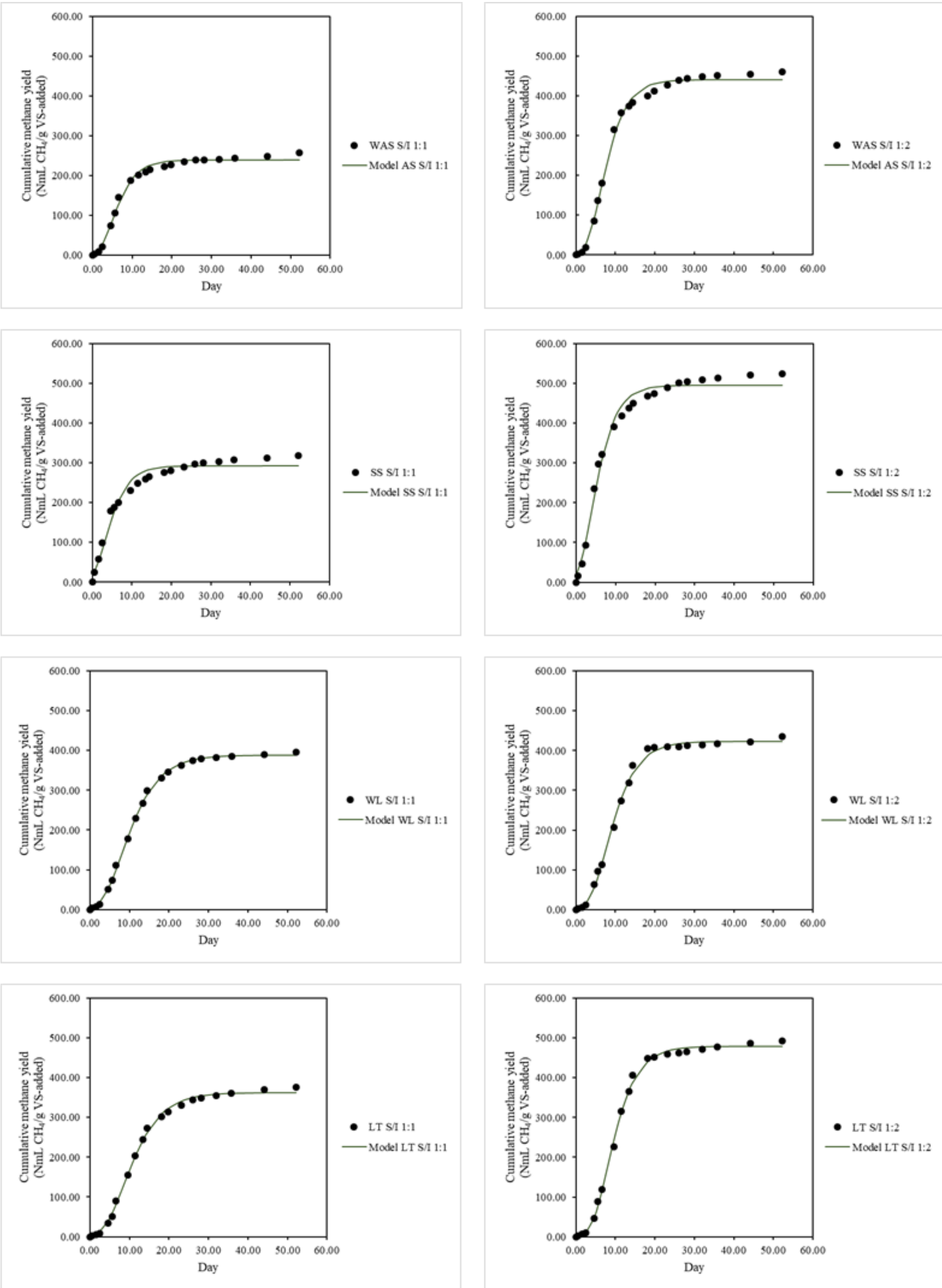

Figure 3.1. Plot of maximum methane yield (G_0) from Gompertz model and experimental methane yield of single substrate experiment (WAS: waste activated sludge; SS: Swine slurry- WL: water lily; LT: lotus)

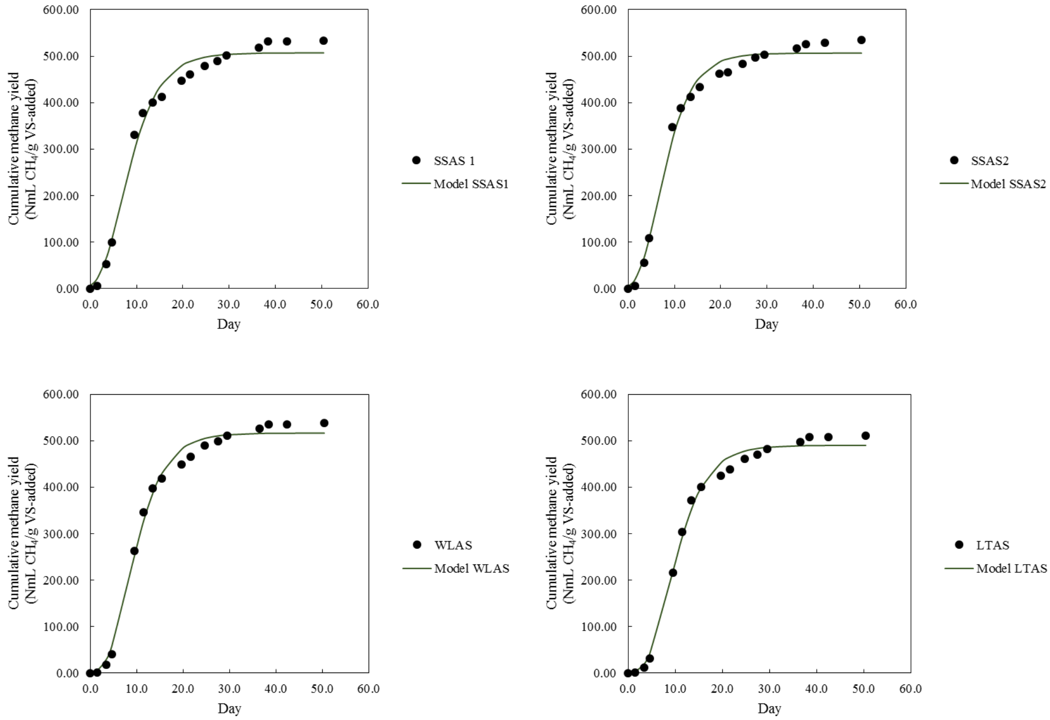

Figure 3.2. Plot of maximum methane yield (G_0) from Gompertz model and experimental methane yield of co-digestion experiment. (SSAS 1: Swine slurry- waste activated sludge 1:1; SSAS 2: Swine slurry- waste activated sludge 1:2; WLAS: water lily- waste activated sludge; LTAS: lotus-waste activated sludge)

There is a reduction in lag-phase period when WAS co-digested with swine slurry. However, it is not significantly different (p>0.05). In case of co-digestion of WAS and plant biomass, there is significant increase in lag phase period. The combination of WAS properties, which is hard to degrade, and plant biomass might contribute to the longer lag-phase period.

The increase in lag-phase period of co-digestion with plant biomass can be overcome with pre-treatment process. Pre-treatment of substrate can be performed chemically, physically, or biologically. The purpose of pre-treatment is to make the organic matter of substrate more available to be converted by microorganisms (Bjerg-Nielsen et al., 2018). Substrate pre-treatments are known to enhance its degradability thus improving the biogas production.

Experimental methane yield (EMY) of co-digested WAS with different substrates was significantly higher than WAS itself. The increase of methane yield of co-digestion of WAS with SS, WL, and LT are 14.89, 10.97, and 16.89% respectively. This indicated that co-digestion of WAS improve its methane yield. Even though there was an increase in lag-phase period, the methane yield was improved. Previous study on co-digestion of WAS with other materials shows increase methane yields. The co-digestion of municipal sewage sludge with swine manure increase biogas production nearly to 40% when swine manure added by 30% (Borowski et al., 2014). The co-digestion of municipal WAS with *Egeria densa* grass improve its methane potential (Zhen et al., 2015). However, opposite study results by Wang et al. (2014) was observed. Sewage sludge co-digestion with *Eleusine indica* grass negatively affect methane production. In this study, water lily and lotus plant shoot biomass was not adversely affected the methane production.

The increase of methane yield of WAS after co-digestion might be caused by the improvement of its characteristics such as better C/N ratio. Plant biomass contains higher carbon compared with WAS. The C/N ratio balance might improve the anaerobic digestion process through elimination of VFA accumulation and ammonia. (Zhen et al., 2015).

Table 3.5. Synergistic or antagonistic effects (α) produced from co-digestion

Parameters	Substrate Ratio (%VS)	G_0 (mL CH_4/g-VS)	CMP (mL CH_4/g-VS)	α
WAS	100	441.61 ± 13.94	441.61 ± 13.94	
SS	100	495.58 ± 5.35	495.58 ± 5.35	
WL	100	423.61 ± 3.17	423.61 ± 3.17	
LT	100	478.89 ± 6.22	478.89 ± 6.22	
SSAS 1	50% : 50%	507.38 ± 12.37	468.60 ± 9.41	1.10 ± 0.03
SSAS 2	33% : 67%	506.69 ± 8.28	459.42 ± 10.93	1.10 ± 0.02
WLAS	33% : 67%	516.21 ± 5.13	433.24 ± 10.75	1.18 ± 0.02
LTAS	33% : 67%	490.08 ± 19.38	451.48 ± 13.85	1.08 ± 0.03

WAS: wate activated sludge; SS: Swine slurry- WL: water lily; LT: lotus; SSAS 1: Swine slurry- waste activated sludge 1:1; SSAS 2: Swine slurry- waste activated sludge 1:2; WLAS: water lily- waste activated sludge; LTAS: lotus- waste activated sludge; G_0: Maximum methane yield (model); CMP: calculated methane potential

Value represented means ± standard deviation (SD)

$\alpha > 1$; the mixture has a synergistic effect in the final production.

$\alpha = 1$; the substrates work independently from the mixture.

$\alpha < 1$; the mixture has a competitive effect in the final production.

3.4.4. Synergistic effect of substrate co-digestion

Co-digestion of substrates can result in either enhancement or reduction of methane yield. The increase of methane production indicates there is synergistic effect from each substrate meanwhile reduction of methane yield indicates antagonistic effect of individual substrate in co-digestion. Table 3.5 shows the α of co-digestion of waste activated sludge with swine slurry or water lily biomass or lotus biomass. The G_0 value is the maximum methane yield obtained from modeling. CMP value represents calculated methane yield considering the individual substrate ratio in the co-digestion mixture. The CMP is calculated based on the G_0 of individual substrate.

Based on the result in Table 3.5 all co-digestion of waste activated sludge with swine slurry or plant biomass result in synergistic effects ($\alpha > 1$). This means that the co-digestion improves methane production from the waste activated sludge.

The synergism may result from nutrient improvement or physicochemical properties improvement in which single substrate are lack, therefore, increase its methane production (Nielfa et al., 2015). As previously described, waste activated sludge contains the least amount of carbon among other substrates. Addition of swine slurry or plant biomass might increase carbon content in the mixture. Carbon content is important in methane production process.

3.5. Conclusion

The bio-methane potential value of waste activated sludge was range from 255.2 – 468.9 NmL CH_4 /g VS-added. The improvement of methane yield was observed after co-digestion. The biodegradability increase with decrease in S/I ratio. Higher inoculum concentration compared to the substrate is expected to increase the production of methane. Co-digestion of waste activated sludge with swine slurry, water lily, or lotus shoot biomass produce synergistic effect with $\alpha > 1$ indicating that co-digestion improve the methane potential yield.

The maximum cumulative methane yield of substrate co-digestion is more than 500 NmL CH_4 /g VS-added in all substrates. To increase the bio-methane potential of waste activated sludge, swine slurry, water lily, and lotus biomass are potential co-digestion substrate for waste activated sludge anaerobic digestion.

References

Abudi, Z. N., Hu, Z., Sun, N., Xiao, B., Rajaa, N., Liu, C., & Guo, D. (2016). Batch anaerobic co-digestion of OFMSW (organic fraction of municipal solid waste), TWAS (thickened waste activated sludge) and RS (rice straw): Influence of TWAS and RS pretreatment and mixing ratio. *Energy*, *107*, 131-140.

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J. L., Guwy, A. J., ... & Van Lier, J. B. (2009). Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. Water science and technology, 59(5), 927-934.

Apha, A. (2005). WEF, 2005. Standard methods for the examination of water and wastewater, 21, 258-259.

Appels, L., Baeyens, J., Degrève, J., & Dewil, R. (2008). Principles and potential of the anaerobic digestion of waste-activated sludge. *Progress in energy and combustion science*, *34*(6), 755-781.

Bjerg-Nielsen, M., Ward, A. J., Møller, H. B., & Ottosen, L. D. M. (2018). Influence on anaerobic digestion by intermediate thermal hydrolysis of waste activated sludge and co-digested wheat straw. *Waste*

Management, *72*, 186-192.

Bonmati, A., Flotats, X., Mateu, L., & Campos, E. (2001). Study of thermal hydrolysis as a pretreatment to mesophilic anaerobic digestion of pig slurry. *Water Science and Technology*, *44*(4), 109-116.

Borowski, S., Domański, J., & Weatherley, L. (2014). Anaerobic co-digestion of swine and poultry manure with municipal sewage sludge. *Waste management*, *34*(2), 513-521.

Cao, Y., & Pawłowski, A. (2012). Sewage sludge-to-energy approaches based on anaerobic digestion and pyrolysis: Brief overview and energy efficiency assessment. *Renewable and Sustainable Energy Reviews*, *16*(3), 1657-1665.

Chae, K. J., Jang, A. M., Yim, S. K., & Kim, I. S. (2008). The effects of digestion temperature and temperature shock on the biogas yields from the mesophilic anaerobic digestion of swine manure. *Bioresource technology*, *99*(1), 1-6.

Chynoweth, D. P., Turick, C. E., Owens, J. M., Jerger, D. E., & Peck, M. W. (1993). Biochemical methane potential of biomass and waste feedstocks. *Biomass and bioenergy*, *5*(1), 95-111.

Costa, J. C., Gonçalves, P. R., Nobre, A., & Alves, M. M. (2012). Biomethanation potential of macroalgae Ulva spp. and Gracilaria spp. and in co-digestion with waste activated sludge. *Bioresource technology*, *114*, 320-326.

Gunaseelan, V. N. (1997). Anaerobic digestion of biomass for methane production: a review. *Biomass and bioenergy*, *13*(1-2), 83-114.

Honda, S. I., Miyata, N., & Iwahori, K. (2002). Recovery of biomass cellulose from waste sewage sludge. *Journal of Material Cycles and Waste Management*, *4*(1), 46-50.

Kafle, G. K., Kim, S. H., & Sung, K. I. (2013). Ensiling of fish industry waste for biogas production: a lab scale evaluation of biochemical methane potential (BMP) and kinetics. *Bioresource technology*, 127, 326-336.

Kim, Y. S., Yoon, Y. M., Kim, C. H., & Giersdorf, J. (2012). Status of biogas technologies and policies in South Korea. *Renewable and Sustainable Energy Reviews*, *16*(5), 3430-3438.

Ministry of Environment (MOE). 2017. Livestock excreta management http://eng.me.go.kr/eng/web/index.do?menuId=277 access on 18th September 2018. 10.59 pm.

Mottet, A., François, E., Latrille, E., Steyer, J. P., Déléris, S., Vedrenne, F., & Carrère, H. (2010). Estimating anaerobic biodegradability indicators for waste activated sludge. *Chemical engineering journal*, *160*(2), 488-496.

Moukazis, I., Pellera, F. M., & Gidarakos, E. (2018). Slaughterhouse by-products treatment using anaerobic digestion. Waste Management, 71, 652-662.

Muradov, N., Taha, M., Miranda, A. F., Kadali, K., Gujar, A., Rochfort, S., ... & Mouradov, A. (2014). Dual application of duckweed and azolla plants for wastewater treatment and renewable fuels and petrochemicals production. *Biotechnology for biofuels*, *7*(1), 30.

Nielfa, A., Cano, R., & Fdz-Polanco, M. (2015). Theoretical methane production generated by the co-digestion of organic fraction municipal solid waste and biological sludge. *Biotechnology Reports*, *5*, 14-21.

Raposo, F., De la Rubia, M. A., Fernández-Cegrí, V., & Borja, R. (2012). Anaerobic digestion of solid organic substrates in batch mode: an overview relating to methane yields and experimental procedures. *Renewable and Sustainable Energy Reviews*, *16*(1), 861-877.

Raposo, F., Fernández-Cegrí, V., De la Rubia, M. A., Borja, R., Béline, F., Cavinato, C., ... & Ganesh, R. (2011). Biochemical methane potential (BMP) of solid organic substrates: evaluation of anaerobic biodegradability using data from an international interlaboratory study. *Journal of Chemical Technology & Biotechnology*, *86*(8), 1088-1098.

Seo, B. S., Park, C. M., Song, U., & Park, W. J. (2010). Nitrate and phosphate removal potentials of three willow species and a bald cypress from eutrophic aquatic environment. *Landscape and ecological engineering*, *6*(2), 211-217.

Shelton, D. R., & Tiedje, J. M. (1984). General method for determining anaerobic biodegradation potential. *Applied and environmental microbiology*, *47*(4), 850-857.

Sudiarto, S. I. A., Choi, H. L., & Renggaman, A. (2015). Application of Phytoremediation for Total Nitrogen and Total Phosphorus Removal from Treated Swine Wastewater and Bio-methane Potential of the Biomass. 유기물 *자원화*, *23*(4), 21-31.

Sudiarto, S. I. A., Renggaman, A., & Choi, H. L. (2019). Floating aquatic plants for total nitrogen and phosphorus removal from treated swine wastewater and their biomass characteristics. *Journal of environmental management*, *231*, 763-769.

Tel-Or, E., & Forni, C. (2011). Phytoremediation of hazardous toxic metals and organics by photosynthetic aquatic systems. *Plant Biosystems, 145*(1), 224-235.

Tyagi, V.K., Lo, S.-L. 2013. Sludge: A waste or renewable source for energy and resources recovery? *Renewable and Sustainable Energy Reviews*, **25**, 708-728.

Wang, F., Hidaka, T., & Tsumori, J. (2014). Enhancement of anaerobic digestion of shredded grass by co-digestion with sewage sludge and hyperthermophilic pretreatment. *Bioresource technology*, *169*, 299-306.

Weemaes, M.P., Verstraete, W.H. 1998. Evaluation of current wet sludge disintegration techniques. *Journal of Chemical Technology and Biotechnology*, **73**(2), 83-92.

Yoon, Y. M., Kim, S. H., Shin, K. S., & Kim, C. H. (2014). Effects of substrate to inoculum ratio on the biochemical methane potential of piggery slaughterhouse wastes. *Asian-Australasian journal of animal sciences*, *27*(4), 600.

Zhang, W., Wei, Q., Wu, S., Qi, D., Li, W., Zuo, Z., & Dong, R. (2014). Batch anaerobic co-digestion of pig manure with dewatered sewage sludge under mesophilic conditions. *Applied energy*, *128*, 175-183.

Zhen, G., Lu, X., Kobayashi, T., Li, Y. Y., Xu, K., & Zhao, Y. (2015). Mesophilic anaerobic co-digestion of waste activated sludge and Egeria densa: performance assessment and kinetic analysis. *Applied* *energy*, *148*, 78-86.

CHAPTER 4. GENERAL CONCLUSION

Waste activated sludge (WAS) utilization is an important part in waste management practice. Therefore, waste activated sludge treatment and utilization becomes important area of study. Waste activated sludge then should be seen as resource to be utilized for different purposes according to the needs, available technology, and economic benefit of wastewater treatment plants, the community surrounding it, and government regulation.

WAS utilization for land application and anaerobic digestion feedstock are sustainable WAS management option. Sludge application on land and treated wastewater effluent utilization for land application is a common practice in waste management. Proper application of treated wastewater and sludge might improve the soil properties and benefit in plant growth. WAS and treated wastewater contain nutrients beneficial for plant growth such as nitrogen, phosphorus, potassium, and micronutrients. Based on its characteristics, WAS and treated wastewater effluent has lower BOD content compared with raw swine slurry indicating more stable material which will reduce the environmental pollution upon application. However, the application of WAS also poses environmental risk, especially in relation with its salinity indicated by high EC. Waste activated sludge (WAS) and membrane bio-reactor effluent (MBRE) have high EC of 4.95 and 2.4 mS/cm. Higher EC might also affect the seed germination. In addition, high Zn content of 1,589 mg/kg dry matter which is above the permitted limit also observed. The application rate and method should be considered for land application purposes of waste activated sludge

(WAS). It is also observed that MBRE is suitable for irrigation purpose since it has less solid content and considerable amount of nutrients that might have beneficial effect on the plant. In addition, the GI of wheat and radish are higher in MBRE treatment indicating less germination and root elongation inhibition on the plant. However, the dilution of MBRE prior to utilization is important since at 40% concentration it reduces the GI value of radish greatly. At each concentration, there is significant difference ($p<0.05$) of GI between radish and wheat in WAS treatment and starting at 20% in MBRE treatment. This indicates that radish exhibit different response compared with wheat towards WAS at every concentration and at higher concentration of MBRE. At each concentration, there is significant difference ($p<0.05$) of GI between radish and wheat in WAS treatment and starting at 20% in MBRE treatment. This indicates that radish exhibit different response compared with wheat towards WAS at every concentration and at higher concentration of MBRE. The germination assay shows that WAS is phytotoxic start at 5% concentration for radish meanwhile for wheat, the phytotoxicity of WAS is seen at 100% concentration. Treated swine wastewater effluent, MBRE, phytotoxicity was observed at concentration 40% for radish seed and 100 % for wheat seed.

Besides the potential of land application of WAS, the anaerobic digestion of WAS is also promising option The bio-methane potential value of WAS was range from 255.2 – 468.9 NmL CH_4/g VS-added. The improvement of methane yield was observed after co-digestion. The biodegradability increase with the decrease in S/I ratio. Higher inoculum concentration compared to the substrate is expected to increase the production of methane. Co-digestion of waste activated sludge with swine slurry, water lily, or lotus shoot biomass produce synergistic effect with $\alpha > 1$ indicating that co-digestion improve the methane potential yield. To increase the bio-methane potential of waste activated sludge, swine slurry (SS), water lily (WL), and lotus (LT) biomass are potential co-digestion substrate for waste activated sludge anaerobic digestion. The increase of methane yield of co-digestion of WAS with SS, WL, and LT are 14.89, 10.97, and 16.89% respectively. Therefore, co-digestion of WAS with other material with higher organic matter are recommended to improve the methane yield of WAS.

Overall, WAS derived from swine slurry wastewater treatment process have potential for land application and feedstock for anaerobic digestion. It also has potential environmental risk upon land application, however, knowing the potential and limitation might help in how to manage WAS appropriately in the future. This study results provide basic information of WAS derived from swine wastewater treatment characteristics and bio-methane potential which is still limited. Looking at the bigger picture, integrated WAS management with livestock waste treatment would be possible. The WAS produced from livestock wastewater treatment plant can be utilized as a part of feedstock on anaerobic digestion plant. For the land application of WAS, field study on WAS application on cropland is necessary. Field studies will confirm the extent of WAS effect on crops and land which will give better idea. The study on how to improve WAS characteristics will be important, especially in relation with how to reduce the EC and metals content. In the future, further study on swine slurry derived WAS pre-treatment prior to anaerobic digestion would be an interesting topic in order to improve the bio-methane production.

요약 (국문초록)
가축폐수처리 및 잠재적 이용에 따른 폐기물 활성 슬러지의 특성

활성 슬러지 공정은 지방 자치 내, 가축 관련 및 산업 폐수를 처리하는 데 사용되는 가장 보편적인 폐수 처리 공정 중 하나이다. 활성 슬러지 공정은 반응기에 미생물 응집체가 떠있는 유기적 생물학적 처리이다. 돼지 슬러리의 폐수 처리 과정에서 슬러지(대부분이 미생물 응집체)가 생성된다. 생성된 대부분의 슬러지가 낭비되는 반면 일부 슬러지는 시스템을 유지하기 위한 폐수 처리 시스템으로 다시 재활용된다. 폐기물 활성 슬러지(WAS)는 추가적인 환경 오염을 방지하기 위해 처리되어야 한다.

일반적으로 폐기물 활동 슬러지는 소각, 매립, 토지 환원 및 또는 해양 투기를 통해 관리된다. 그러나 1996년 런던 협약에 따라, 2012-2013년부터 한국에서 오니, 음폐수, 가축 폐수의 해양 투기가 금지되었다. 이 정책은 이러한 종류의 폐기물을 추가적으로 처리하고 재활용해야 한다는 결과를 만들어냈다. 따라서, 폐기물 활성 슬러지 처리와 활용은 연구의 중요한 영역이 되었다.

그 연구는 두 부분으로 나뉜다. 첫번째 부분은 WAS 및 멤브레인 생물반응기 유출물(MBRE)의 종자 발아에 미치는 물리화학적 특성과 영향에 대해 연구했다. WAS와 MBRE는 분리와 침전 후 돼지 폐수 처리

과정에서 나온 제품이다. 종자 발아 검사는 WAS와 MBRE의 약해를 평가하기 위해 무와 밀 씨앗을 이용하여 실시 되였다. 그 다음 발아지수(GI)는 뿌리의 연장 및 WAS와 MBR의 다양한 농도에 노출되었을 때 발아되는 종자의 수에 근거하여 종자 발아 검사를 통해 결정된다. 본 연구의 결과, 토양 환원을 위한 WAS와 MBRE의 잠재적 활용에 대해 논의되었다. 연구의 두 번째 부분은 잠재 바이오메탄(BMP) 측정을 통한 혐기성 소화 공급원료로서의 WAS 활용에 초점을 맞추었다. 또한, 돼지 슬러리(SS), 수련(WL) 바이오매스, 또는 연꽃(LT) 바이오매스로 WAS의 통합 분해도 조사되었다.

첫 번째 연구에 따르면, WAS와 MBRE는 생화학적 산소 요구량(BOD5) 수준에 따라 생 돼지 폐수보다 생물학적으로 더 안정적이다. WAS와 MBRE의 BOD5는 각각 804와 376으로, BOD5가 20,563인 원 돼지 슬러리보다 훨씬 적다. 폐기물 활성 슬러지는 식물에 유익한 다량원소(N, P, K)와 미량원소(Na, Mg, Fe, Ca, Cu, Zn, Mo) 또한 포함하고 있다. 단, WAS는 각각 357과 1,589 mg/kg인 Cu와 Zn의 건물량을 포함하며, 이는 토지 환원을 위해 고려되어야 한다. Cu 농도는 표준 시비 한계치에 매우 근접한 반면 Zn 농도는 한국 농림부의 한계치보다 훨씬 높다. MBRE와 비교하면 Cu 및 Zn 농도는 한계치(0.03 및 0.14 mg/L)보다 훨씬 낮다. WAS와 MBRE의 전기전도도(EC)는 각각 4.9mS/cm와 2.4mS/cm으로 높아 보인다. EC는 염도와 관련이 있다. WAS와 MBRE의 높은 EC는 장기적으로 사용되었을 때 농지의

2차적 염료화 작용에 기여할 수 있다. 발아 지수(GI) 값이 80% 초과 시 비식물독성으로 간주되는 반면, 50% 미만은 매우 독성이 강하며 농업 사용에 적합하지 않은 것으로 간주된다. 종자 발아 검사에 따르면, WAS는 5% 농도부터 무에 약해를 보인다. MBRE의 경우, 40% 농도에서 약해가 관찰되었다. 밀의 경우, WAS와 MBRE는 100% 농도에서 약해를 보인다.

WAS의 토지 환원은 WAS 잠재적 활용의 한 가지 방안이다. 토지 환원 외에도 혐기성 소화 또한 WAS 관리의 지속 가능한 방안으로 간주된다. 혐기성 소화를 통해 메탄(CH_4) 가스 형태의 재생 에너지를 얻을 수 있다. 이 두 번째 연구에서는 BMP 배치 검사가 수행되었다. 돼지 슬러리(SS)와 식물 바이오매스로 WAS의 통합 분해 또한 조사되었다. WAS는 에너지 생산 잠재력을 감소시킬 수 있는 유기물을 덜 함유하고 있다. 따라서, 많은 유기물 기질과의 통합 분해는 바이오가스의 잠재력을 증가시키는 한 가지 방법이다. 폐기물 활성 슬러지의 잠재 바이오메탄 값의 범위는 255.2에서 468.9 NmL CH_4 /g VS-added까지이다. 돼지 슬러리(SS), 수련 및 연꽃 식물바이오매스는 WAS에 비해 유기물 함량이 높다. 돼지 슬러리, 수련 및 연꽃 싹의 바이오매스와 함께 폐기물 활성 슬러지를 통합 분해하면 $\alpha > 1$의 시너지 효과가 나타나 통합 분해가 메탄 잠재 산출량을 높인다는 것을 알 수 있다. 기질 통합 분해의 최대 누적 메탄 산출량은 모든 기질에서 500 NmL CH_4 /g VS-added보다 많다. SS, WL 및 LT와의 WAS

통합 분해의 메탄 산출량의 증가율은 각각 14.89, 10.97, 16.89%이다. 폐기물 활성 슬러지의 혐기성 소화는 전기로 전환 가능한 결과물로서 재생 에너지를 생산한다. 메탄 산출량 증가는 SS, WL 및 LT와의 통합 분해에서 관찰되었다. WAS 혐기성 소화의 메탄 산출량을 증가시키려면 WAS통합 분해를 많은 유기물 기질과 함께 하는 것을 권장한다.

핵심 단어: 폐기물 활성 슬러지, 돼지 폐수, 종자발아, 토지 환원, 잠재 바이오메탄, 식물 바이오매스, 통합 분해

학생 번호: 2014-31451

ACKNOWLEDGMENT

I would like to express my gratitude to everyone who has assisted or made the completion of my research and thesis is possible. First and foremost, I want to express my gratitude to all the members of my advisory committee: Professor Cheol Heui Yun, Professor Myunggi Baik, Professor Cheorun Jo, Professor Hong Lim Choi, and Dr. Dong Yoon Choi. I especially thank to thank Professor Hong Lim Choi for his support throughout my doctoral program. He has provided me opportunities to learn and doing research throughout my doctoral program.

I am also like to give great appreciation to my beloved husband, Mr. Anriansyah Renggaman whose great support and invaluable suggestions are essential to the accomplishment of this doctoral thesis. I also want to thank Animal Environment and Bioengineering laboratory members, especially Mr. Andi Febrisiantosa and Dr. Joonhee Lee for their support and help throughout the program. I would also like to thank Mr. Myung Dong Kim for his great field assistance and making the experimental site an enjoyable workplace.

I thank the Seoul National University (SNU) Lecture & Research Scholarship, SNU Merit-based Scholarship and Cargill Inc. for their financial support during my doctoral program. I also thank the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, and Forestry (iPET), Ministry of Agriculture, Food, and Rural Affairs Republic of Korea for partially funding the research.