공학박사학위논문

Anaerobic digestion for the treatment of heavy metal-containing crop residues

중금속 함유 식물체부산물의 처리를 위한 혐기성소화에 대한 연구

2016 년 8 월

서울대학교 대학원

건설환경공학부

이 종 근

Anaerobic digestion for the treatment of heavy metal-containing crop residues

by

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A dissertation submitted in partial fulfillment
of the requirements for the degree of

Doctor of Philosophy

Department of Civil and Environmental Engineering
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August 2016

ABSTRACT

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Due to endogenous contaminants, treatment methods of crop residues from contaminated sites must be carefully selected considering contaminant separation, environmental impact, and economical concerns. Contaminated residues are generally disposed of by composting, pyrolysis, direct disposal, incineration, ashing, and anaerobic digestion. Anaerobic digestion is a biological process in which microorganisms degrade organic matter and convert into biogas as the end product. Agricultural crop residues are an important source of biomass that can be utilized as a substrate in anaerobic digestion. Anaerobic digestion for crop residues has been applied

as an effective technology in terms of renewable energy production, byproduct utilization, and agricultural waste reduction. For these reasons, anaerobic digestion could be the appropriate option for crop residues from heavy metal contaminated sites with considerations in terms of the aforementioned categories (i.e., contaminant separation, environmental impact, and economical concerns) among various treatment methods. However, heavy metals have been known to adversely affect the anaerobic digestion process, and the fate and effect of heavy metals in crop residues during anaerobic digestion needs to be addressed.

Firstly, biochemical methane potential (BMP) tests using sunflowers (i.e., *Helianthus annuus*) harvested from four differential levels of heavy metals containing soils were conducted to investigate the applicability of anaerobic digestion for heavy metal containing crop residues. According to the results, the methane gas production of crop residues from heavy metals containing soils were comparable to that of the control test, which was not contaminated with heavy metals. Significant adverse effects of heavy metals in crop residues on methane gas production were not observed under the experimental conditions of this study. Even though anaerobic bacterial activities are known to be typically affected by the amounts of heavy metals in the form of liquid phase, all of the observed amounts of heavy metals in this study were not only similar between the test conditions but also below the reported inhibitory levels. These findings revealed that anaerobic digestion can be an alternative to the treatment method of heavy metal-containing crop residues from phytoremediation sites.

In order to investigate the long-term stability on the performance of the anaerobic digestion process, a laboratory-scale continuous stirred-tank reactor (CSTR) was operated for 1,100 days with sunflower harvested in a heavy metal contaminated site. Changes of microbial communities during digestion were identified using pyrosequencing. According to the results, soluble heavy metal concentrations were lower than the reported inhibitory level and the reactor performance remained stable up to OLR of 2.0 g VS/L/day at HRT of 20 days. Microbial communities commonly found in anaerobic digestion for cellulosic biomass were observed and stably established with respect to the substrate. Thus, the balance of microbial metabolism was maintained appropriately and stability on the performance of the anaerobic digestion was confirmed by long-term operation of laboratory-scale CSTR operation.

Although the applicability and stability of anaerobic digestion for heavy metal containing crop residues were ascertained with the conducted tests, inconsistency between biodegradation ratio of biomass and releasing characteristics of heavy metals through biodegradation of biomass was observed. For better understanding of anaerobic digestion of crop residues from heavy metal phytoremediation sites without the adverse effects of heavy metals, the releasing characteristics of endogenous heavy metals should be considered for stable anaerobic digestion process. This study was conducted to examine the releasing characteristics of heavy metals from biomass and the fate of heavy metals after release. According to the volatile solids and carbon balance analyses of anaerobic batch test results, maximum of 60% by wt. of biomass

was degraded. During the biodegradation, among Cd, Cu, Ni, Pb, and Zn, only Cu and Zn were observed in soluble form (approximately 40% by wt. of input mass). The results concluded the irrelevancy between degradation ratio of biomass and ratio of released heavy metals amounts from biomass. It was shown that this discordance was caused by the fate (i.e., precipitation and adsorption) of heavy metal species in solutions after being released from biomass. Thus, ultimate heavy metal concentrations in solutions, which can exert adverse effects on anaerobic digestion performance, were strongly dependent upon not only released heavy metal amounts but also their fate in solution after release.

A model of the anaerobic digestion process which attempts to explain the complex patterns of the anaerobic digestion process is required to better understanding and design anaerobic digestion process. Mathematical models have provided an understanding of important inhibition patterns and have given guidelines for operation and optimization of anaerobic digesters. However, a mathematical model for prediction in change of heavy metal concentrations in anaerobic digestion process according to the degradation of heavy metal containing biomass has not been studied in previous research. For this reason, developing a mathematical model is needed for better understanding of anaerobic digestion of crop residues from heavy metal phytoremediation sites without the adverse effects of heavy metals. In this study, to simulate the change of soluble heavy metals in anaerobic digestion system, a mathematical model based on mass balance is developed. The model can describe the

soluble heavy metal concentrations in anaerobic digester according to degradation of heavy metal containing crop residues. From the sensitivity analysis for the variables used in the model, OLR has the highest sensitivity with gradient of trend line. Although substrate degradation kinetic (k) has relatively low sensitivity to the change of heavy metal concentrations in liquid phase, the k value can be an important input parameter due to its variation with type of substrate. The developed model will provide useful information on anaerobic digestion process design for heavy metal containing substrate and will expand the substrate types using simple batch test for substrate degradation kinetics. Several application examples and required improvements were also discussed. However, the model developed in this study includes several uncertain assumptions for the convenience of calculation (i.e., MLSS is constant during digestion, heavy metal adsorption occurs only to MLSS, etc.). Consequently, upgrading the developed model should be accompanied by verification and improvement of the uncertain assumptions for degree of completion.

Keywords: Anaerobic digestion; Heavy metal-containing crop residues; Treatment of crop residues; Biodegradation; Releasing characteristics of endogenous heavy metal; Model development

Student Number: 2011-20995

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CHAPTER 1
INTRODUCTION

1.1 Background

Phytoremediation is emerging as a cost-effective green technology that utilizes plants to clean up contaminated areas (Salt et al., 1995). After plants are harvested, highly contaminated residues (i.e., plant biomass) must be disposed, and thus, a suitable biomass treatment method needs be considered. Contaminated residues are generally disposed of by composting, pyrolysis, direct disposal, incineration, ashing, and anaerobic digestion (Bridgwater et al., 1999; Kumar et al., 1995; Raskin et al., 1997; Sas-Nowosielska et al., 2004). Among these various treatment methods, the appropriate option should be selected with considerations in terms of the environmental, economical, and energy recovery potential aspects.

Anaerobic digestion for crop residues has been applied as a plausible solution in terms of renewable energy production, byproduct utilization, and agricultural waste reduction (Zhang et al., 2013). When crop residues are harvested from contaminated sites, abandoned lands may be efficiently utilized along with the benefit of remediating contaminated soils (Evangelou et al., 2012). However, anaerobic digestion of crop residues from heavy metal contaminated sites must be approached carefully due to endogenous heavy metals in crop residues.

Existence of heavy metals in the anaerobic digestion process may cause adverse effects, resulting in toxicity to microbial activities and causing possible process upset or failure. The adverse effects (i.e., toxicity) of heavy metals is attributed to interruption of enzyme function and structure due to formation of metal complex with thiol and other groups on protein molecules or replacement of naturally occurring metals in enzyme prosthetic groups (Vallee and Ulmer, 1972). Previous researches have reported that various factors such as soluble metal concentration (i.e., ionic form in the solution), types of metal species, and amount/distribution of biomass in the digester can cause metal inhibition (Bertin et al., 2012; Fang and Chan, 1997). However, there are few studies on the effects of endogenous heavy metals within substrate. The effects of heavy metals in crop residues on anaerobic digestion process should be studied to secure sustainable application of anaerobic digestion for heavy metal-containing crop residues.

1.2 Objectives

The primary objective of this study is to evaluate anaerobic digestion as a treatment method for crop residues from heavy metal contaminated sites.

The specific objectives of this study are:

1) Assessing the stability of heavy metal containing crop residues for application of anaerobic digestion as a treatment method
2) Investigating the fate of heavy metals that are released from crop residues during anaerobic degradation
3) Developing a prediction model of heavy metal concentrations in relation to biomass for stable anaerobic digestion process

1.3 Dissertation structure

This dissertation consists of 7 chapters (Fig. 1.1): Following this introduction in chapter 1, chapter 2 presents the literature review. It presents the various treatment methods of crop residues and selects an appropriate method for heavy metal-containing crop residues, basics of anaerobic digestion for cellulosic biomass, and effects of heavy metals on anaerobic digestion. Chapters 3 and 4 describe the feasibility and stability of

anaerobic digestion for heavy metal-containing crop residues harvested from contaminated sites. The applicability of anaerobic digestion as a treatment method of heavy metal containing crop residues was investigated using biochemical methane potential (BMP test) in chapter 3. Chapter 4 presents the effects of endogenous heavy metals on reactor performance stability with a laboratory-scale continuous stirred-tank reactor (CSTR) and response microbial communities. The releasing characteristics of heavy metals from crop residues and the fate of heavy metals that are released from crop residues during anaerobic degradation are discussed in chapter 5. In chapter 6, a prediction model of heavy metal concentrations was developed and suggested. Chapter 7 gives a summary of the entire dissertation, conclusions and recommendations.

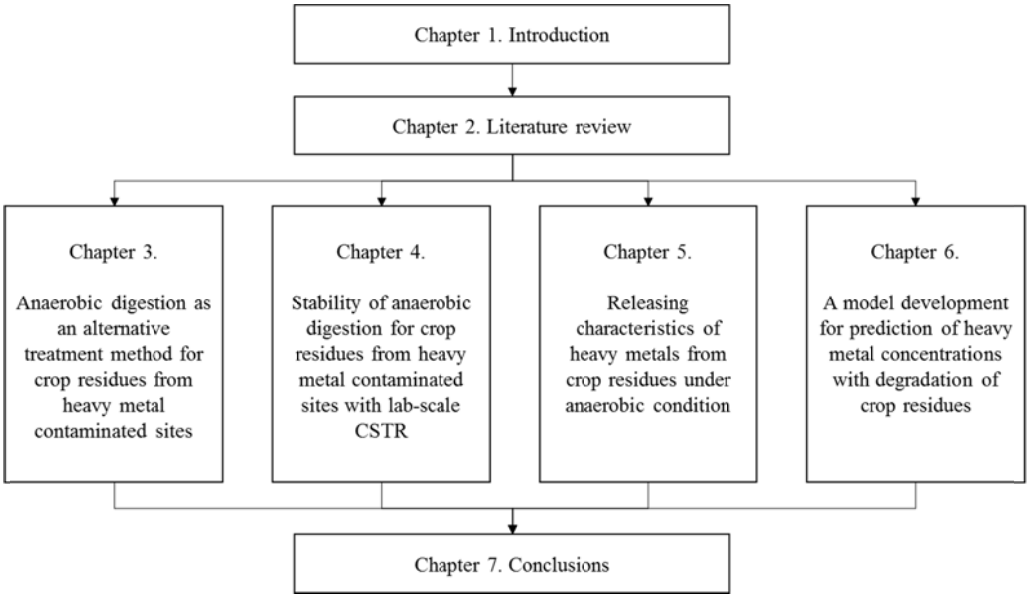

Figure 1.1 Structure of dissertation

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CHAPTER 2
LITERATURE REVIEW

2.1 Treatment methods of heavy metal-containing crop residues[1]

For treatment of contaminated crop residues, several methods are described in many previous studies. Fig. 2.1 shows the treatment methods of plant that have been used to harvest from heavy metal contaminated sites. The composting, compaction and pyrolysis are treated as pretreatment steps, because considerable amount of contaminants will still exist after each of the process.

After generation of crop residues, it is important to reduce the volume of produced crop residues (Salt et al., 1995b, McGrath, 1998 and Blaylock & Huang, 2000) and to remove excess water content. Volume reduction of crop residues can be achieved by composting, compaction or pyrolysis. Comparison of pretreatment options is shown in Table 2.1. This improves the technical parameters and reduces the cost of transportation to the treatment or disposal site.

[1] Significant portions of this part were extracted and rearranged form Sas-Nowosielska et al., " Phytoextraction crop disposal-an unsolved problem", Environmental Pollution, 128, 373-379.

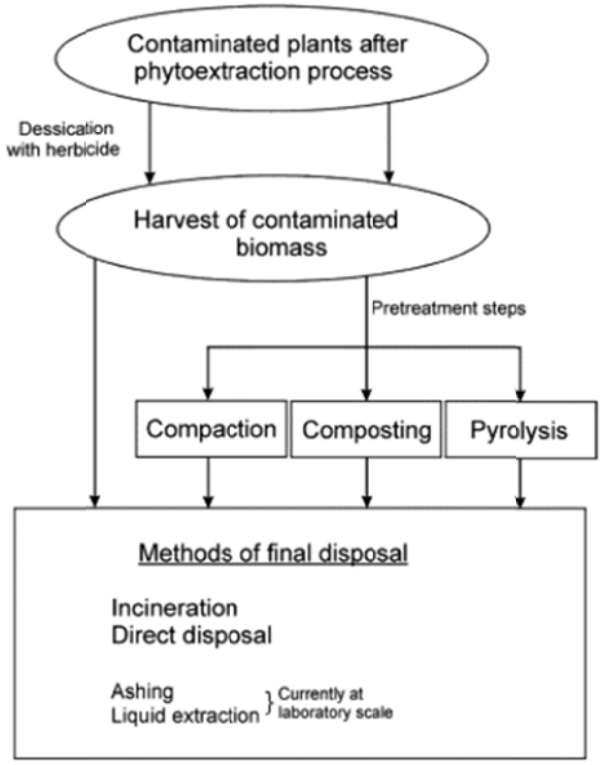

Figure 2.1 The most commonly proposed treatment methods of contaminated crop residues (Source: Sas-Nowosielska et al., 2004)

Composting has been proposed as a post-harvest crop treatment by some authors (Kumar et al., 1995, Salt et al., 1995b, Salt et al., 1998, Blaylock & Huang, 2000 and Garbisu & Alkorta, 2001). Hetland et al. (2001) conducted lab-scale experiments with plant biomass (i.e., sunflower, grass) contaminated with lead obtained after induced phytoremediation. The disintegrated plant biomass (diameter: less than 0.16 cm) was composted in 125 mL glass bottles with constant aeration for two months. About 25%

of by wt. (dry basis) loss was reported. Soluble organic compounds that enhanced lead solubility formed by composting process was observed with leaching tests of composed material. These results emphasized that composting can significantly reduce the volume of harvested biomass, however lead-containing plant biomass would still require post-treatment process before final disposal. Chemical additives are commonly used as a chemical agent for improve the efficiency of phytoremediation process (Blaylock et al., 1997, Salt et al., 1998 and Epstein et al., 1999). Sarret et al. (2001) have documented that metal and chelate complexes taken up by Phaseolus vulgaris and accumulated in shoots can be totally dissociated in the case of zinc and only partly dissociated in the case of lead. It seems that highly mobile and leachable metal and chelate complexes can be contained in plant biomass harvested after induced phytoremediation. Vassil et al. (1998) reported that complex of lead and chelate is taken up by Indian mustard plants and accumulated in the part of shoots. Moreover Perronet et al., 2000 and Zhao et al., 2000 also showed that most of Zn within the leaves of hyperaccumulating plant is also present soluble forms. It means that composting process for crop residues from phytoremediation sites should be conducted carefully in order to avoid non-desirable leachates.

Table 2.1 Methods for pretreatment (Source: Adapted from Sas-Nowosielska et al., 2004)

Process types	Costs of transportation In situ-no costs ($/t/km)	Costs of site preparation ($/m^2)	Costs of leachate utilization ($/t)	Costs of processing ($/t)	Advantages	Disadvantages
Composting	1.14-2.28	3.42-5.70	-	11.4-28.5	Volume reduction	Time consuming (2-3 month) Requiring special equipment End product as hazardous waste
Compaction	1.14-2.28	3.42-5.70	153.9	N.A.*	Volume reduction Metal recovery	Requiring special equipment End product as hazardous waste (remaining biomass, leachates)
Pyrolysis	1.14-2.28	-	-	N.A.	Volume reduction (significant) End-product (pyrolytic gas)	End product as hazardous waste (coke breeze)

*N.A.: Not Available

It is necessary to emphasize that the purpose of composting is to reduce the volume and weight of plant material for final disposal, with no consideration to the agricultural properties of the final product. Total dry weight degrade of crop residues is a merit of composting as pretreatment step. It will lower costs of transportation to a hazardous waste treatment facility and costs of deposition or costs of transportation to other facilities, where final treatment method for crop residues will take place. However, approximate 2 to 3 months required for composting, extending time from harvesting to a final disposal. Furthermore, contaminated remained biomass should be treated as hazardous material.

Compaction of harvested plant material was proposed by several researchers (Salt et al., 1995b and Blaylock & Huang, 2000) for processing metal rich crop residues. The process of compaction uses a container equipped with a press and a leachate collection system. Aforementioned, the leachate generated by pressing contaminated crop residues will contain high concentrations of metal and chelate complexes or soluble heavy metal forms (Perronet et al., 2000, Zhao et al., 2000 and Sarret et al., 2001). The leachate should be collected separately and treated appropriately due to contaminants in leachate. Advantages of compaction are close to composting. Shorter time is needed for compaction of the same amount of biomass than composting, depending on efficiency of facility (e.g. volume of container). However, contrast to composting is lack of information on compaction. End-products of compaction (i.e., remaining contaminated biomass, leachates) should be treated as hazardous material.

For further study, research should be conducted to assess how volume and weight of fresh biomass is reduced by compaction depending on plant types (Sas-Nowosielska et al., 2004). Composition and concentration of heavy metals in the leachate generated by pressing should be investigated, as well as method of recycling of recovered metals from solutions.

Pyrolysis also needs post-treatment process, but more significant volume and mass reduction of contaminated crop residues is achieved compared to composting (Bridgwater et al., 1999). Pyrolysis decomposes material under anaerobic conditions and moderate temperatures. Reduction of emission can be achieved by the completely hermetic process. Useful products (i.e., pyrolytic gas, coke breeze) can be obtained during pyrolysis as final product. Heavy metals form contaminated biomass will be contained in coke breeze. It means that this product should be treated as hazardous waste and dispose at hazardous waste dumping site. On the other hand, coke breeze could be used in a lead/zinc smelter instead of coke, and then lead or zinc might be recovered during smelting process. The limitations in the pyrolysis of plant material would be the maximum moisture limit (30%) and the very high costs of installation and operation if used solely for plant treatment.

The incineration process destroys organic matters in crop residues, releasing endogenous metals, mainly as oxides. The liberated metals are entrained in the slag or released to the effluent gases. Modern flue gas-cleaning technology assures effective capture of the metal-containing dust. Plant material also can be incinerated in an

incineration plant. Cost of incineration of one ton of hazardous material ranges from 205 to 250 $/t (Table 2.2). To decrease the amount of crop residues to be transported to an incineration facility, desiccation can be used. Preharvest desiccation can be accomplished by treating part of shoots with an herbicide such as glyphosate (Ellis et al., 1998 and Bennet & Shaw, 2000). This treatment method also reduces the likelihood of leachate production from the crop residues during harvest and transport. Additional risks connected with the use of glyphosate is negligible due to its common usage and quickly degradation characteristic in soil with very low toxicity to soil organisms. In addition, concentration used for desiccation can be lower than concentration recommended by manufacturer. Reducing by more than 90% by wt. (dry basis) of contaminated biomass is an advantage of this method.

The volume/weight of harvested crop residues also can be reduced by ashing. This option for contaminated crop disposal is often mentioned (Kumar et al., 1995, Salt et al., 1995b, Salt et al., 1998, Blaylock et al., 1997, Dushenkov et al., 1997, Cunningham & Berti, 2000 and Garbisu & Alkorta, 2001), but lack of data on its application is a problem. Hetland et al. (2001) determined the possibility of co-firing crop residues with sub-bituminous coal in a down-fired combustion system designed for lab-scale experiments. They reported that co-firing crop residues with coal reduced the mass of lead-contaminated plant material by over 90% and distributed lead into the ash. These results revealed that ashing could be a feasible method of biomass reduction, but more data on combustion systems and ash disposal is necessary. It may be possible to recycle

the recovered metals from the ash, however there are no estimates of the economical aspect or feasibility of such a process (Kumar et al., 1995, Salt et al., 1995b, Salt et al., 1998, Raskin et al., 1997, Blaylock et al., 1997, Dushenkov et al., 1997, Cunningham & Berti, 2000 and Garbisu & Alkorta, 2001).

Although direct disposal of contaminated crop residues as hazardous waste is the least complicated approach of disposal, deposition of crop residues at a hazardous waste site is costly (Sas-Nowosielska et al., 2004). In addition, these deposition of crop residues at a hazardous waste site is a reverse trend material recycle movement in waste management field.

The use of leaching to extract heavy metals from harvested crop residues has been described (Salt et al., 1995b). Hetland et al. (2001) evaluated chelation extraction as a technique for the recovery of lead from harvested biomass. They examined two chelating agents: EDTA and N-(2-acetamido)iminodiacetic acid (ADA). They observed that at a pH of 4.5 and a 1:4.76 molar ratio of lead to EDTA, it is possible to extract 98.5% of the lead present in the biomass using two sequential batch extractions. In their opinion, this technique would be very attractive if lead could be efficiently and cost-effectively separated from the chelating agent and the chelating agent could then be recycled (Hetland et al., 2001). The residual biomass solids would not need to be disposed of as hazardous waste, because Hetland et al. (2001) shown that it can be calculated that plant material with 2000 mg/kg lead will remain after extraction only 30 mg/kg lead in dry weight. Such materials can be disposed of as municipal wastes.

Although several technologies exist which can remove metals from the solutions, a production-scale process for this type of metal recovery and recycling has yet to be demonstrated (Mulligan et al., 2001).

Table 2.2 Comparison of methods for treatment of contaminated crop residues (Source: Adapted from Sas-Nowosielska et al., 2004)

Process types	Costs of transportation ($/t/km)	Costs of processing ($/t)	Advantages	Disadvantages
Incineration	1.14-2.28	205.2-250.8	Recovery of metals Significant reduction of biomass	Large quantities of polluted exhaust gases High costs
Direct disposal at hazardous waste site	1.14-2.28	153.9-1,295.0	Time effectiveness	High costs Limitation of dumping sites Trend towards material recycle movement in waste management field Slow reduction of contaminated biomass
Ashing	1.14-2.28	N.A.*	Recovery of metals Significant reduction of biomass	Lack of technology development
Liquid extraction	1.14-2.28	N.A.	Recovery of metals	Lack of technology development

*N.A.: Not Available

2.2 Anaerobic digestion of cellulosic biomass[2]

Anaerobic digestion is a plausible dual-purpose technology for treating complex biomass wastes and converting organic matter into biogas, which mainly consists of methane and carbon dioxide with traces of other impurities, such as hydrogen sulfide, ammonia, and water vapor. Because of its advantages over conventional fossil-derived resources, anaerobic digestion has been adopted and integrated into society over the last century, with thousands of full-scale plants currently in operation worldwide. Anaerobic digestion is suitable for converting non-sterile, diverse, complex feedstock into energy-rich biogas. Many biodegradable feedstock such as industrial wastewater, food wastes, animal manure, agro-wastes, sewage sludge, organic fraction of municipal solid waste, among others, have been employed as substrates for commercial biogas production. Such facilities illustrate the unique potential for bioremediation and waste stabilization with concurrent bioenergy production. More recently, cellulosic biomass, namely agro-residues and energy crops, have been gaining much attention as candidate feedstock for producing bioenergy and biobased products. Unlike conventional biological renewable feedstock (i.e., sugar- and starch based crop residues), cellulosic biomass do not directly compete with food or feed production. Moreover, high biomass

[2] Significant portions of this part were extracted and rearranged form Sawatdeenarunat et al., "Anaerobic digestion of lignocellulosic biomass: Challenges and opportunities", Bioresource Technology, 178, 178-186 and Weiland "Biogas production: current state and perspectives", Applied Microbiology and Biotechnology, 85, 849-860.

yields even under low inputs of energy, water, fertilizers, and pesticides, make these crops ideal for biogas (and bioenergy) production (McKendry, 2002).

2.2.1 Principle of anaerobic digestion of biomass

Anaerobic digestion is the naturally occurring, biological pretreatment of organic substrates carried out by robust, mixed culture microbial communities in the absence of oxygen (Khanal, 2008). The consortium of microbes works synergistically to deconstruct recalcitrant biomass structures (like lignocellulose) into their respective fundamental components (Fig. 2.2).

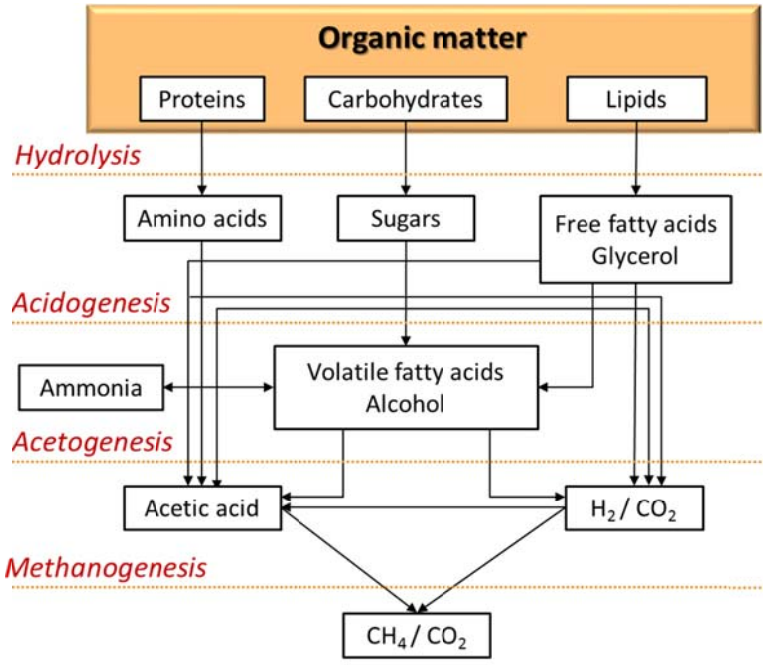

Figure 2.2 Conceptual diagram of anaerobic digestion for biomass

In conventional bioprocessing strategies, the whole cellulosic feedstock is ground and fed into an anaerobic bioreactor to convert complex carbohydrates and organic matter into energy-rich biogas (Weiland, 2010). Though effective, this an insightful study conducted by Yue et al. (2010) suggested that certain microorganisms present in the anaerobic digestion slurry may prefer specific biomass constituents over others. In particular, the authors found that the heterogeneous polysaccharide, hemicellulose, was broken down and metabolized before other structural components. By carefully adjusting the solids retention time (SRT), among several other operating conditions, the anaerobic digestion process may have the ability to promote methane (CH_4) production from hemicellulose exclusively, while leaving behind cellulose and lignin in the fibrous solid residue. The removal of hemicellulose effectively destabilizes the recalcitrant biomass structure, thus allowing for the solubilization (i.e., saccharification) of cellulose by commercial enzymes in the downstream processes (Maclellan et al., 2013; Yue et al., 2011). Glucose, derived from the hydrolysis of cellulose, can serve as a substrate for producing drop in biofuels via the carboxylate platform (Agler et al., 2011) or as a precursor for high-value products such as bioplastics, succinic acid, fungal protein, etc. (FitzPatrick et al., 2010; Cherubini and Strømman, 2011). The organic acids produced through fermentative processes (where applicable) also have potential use in a number of chemical industries and products (e.g., resins, pesticides, fertilizers, etc.) (Cherubini and Strømman, 2011). Any lignin remaining in the solid residue has little commercial value in current markets, but can be burned for in-house heat and

electricity generation. Unique to an anaerobic digestion biorefinery approach, in contrast to conventional biofuel/bioenergy production, is the inherent generation of digestate (i.e., the nutrient-rich residue) resulting from the digested slurry. The digestate has important land-use applications and serves to improve nutrient retention in soil. The idealized anaerobic digestion biorefinery, as illustrated in Fig. 2.3, is a rapidly emerging concept that can significantly improve the commercial viability and applicability of the anaerobic digestion process.

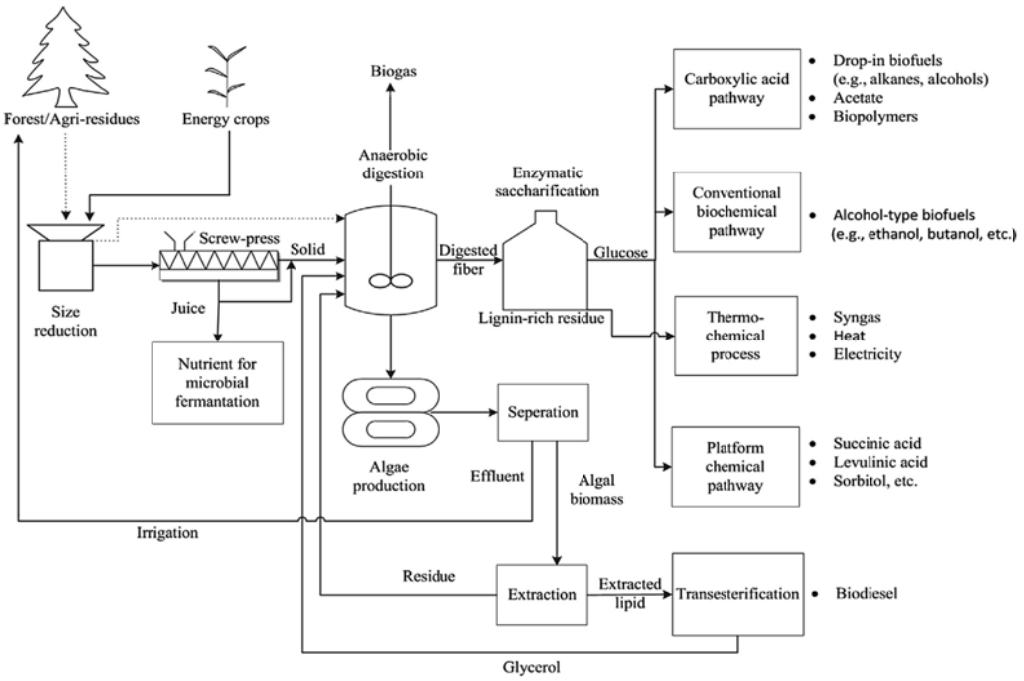

Figure 2.3 The schematics of integrated process for producing biogas and biobased products from cellulosic biomass (Source: Sawatdeenarunat et al., 2015)

2.2.2 Structure and composition of cellulosic biomass

Cellulosic biomass is an abundantly available resource with an annual (global) yield of over 200 billion dry metric tons per year (Kumar et al., 2008). For example, U.S. alone produces about 1.37 billion dry tons of such biomass per year for biofuel production (Limayem and Ricke, 2012). Common examples of these renewable resources include agricultural and forest residues, and dedicated energy crops (Cherubini, 2010). As shown in Fig. 2.4, the basic structure of lignocellulose is comprised primarily of cellulose (35–50%), hemicellulose (20–35%), and lignin (10–25%) (Liu et al., 2008), along with smaller quantities of other organic and non-organic compounds like proteins, lipids, and other extractives (Frigon and Guiot, 2010). Table 2.3 summarizes the typical composition of some commonly used cellulosic feedstock. It is prudent to mention that the amounts of these constituents not only varies between species, but can also vary due to growth conditions and maturation. Cellulose is the main constituent of virtually all plant cell walls, thus making this compound one of the most abundant (renewable) polymers on the planet. Hemicellulose, in contrast, is a highly branched heteropolysaccharide consisting of a wide variety of sugars (C5 and C6). The side groups extending off of the main hemicellulosic backbone preclude the polymer from forming crystalline structures reinforced by hydrogen bonding, unlike cellulose. The individual sugars of hemicellulose can differ considerably depending on the plant species, however, in general, the saccharification of hemicellulose typically produces a mixture of glucose, galactose, mannose, arabinose, xylose, and rhamnose.

The last main constituent of lignocellulose, namely lignin, is a phenylpropane-based polymer with little value for bioenergy production, despite being the second most abundant polymer on the earth. Lignin is an essential part of the biomass structure as it provides mechanical support and water impermeability to the secondary cell walls of plants, but lignin also serves as both a physical and biochemical barrier that impedes most biomass-to-bioenergy conversion processes.

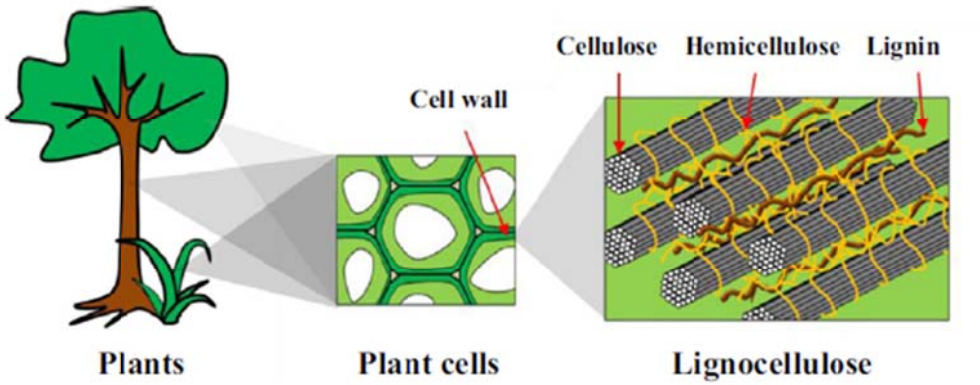

Figure 2.4 Structure of cellulosic biomass in plant cell walls

Table 2.3 The characteristic of selected cellulosic biomass (Source: Adapted from Sawatdeenarunat et al., 2015)

Biomass types	Cellulose (%)	Hemicellulose (%)	Lignin (%)	C/N ratio	References
Corn stover	37.5	22.4	17.6	63	Karthikeyan and Visvanathan (2012) and Li et al. (2014)
Wheat straw	38.2	21.2	23.4	60	Karthikeyan and Visvanathan (2012)
Switch grass	31.0–45.0	20.0–31.0	12.0-18.0	90	Brown et al. (2012)
Bagasse	38.2	27.1	20.2	118	Karthikeyan and Visvanathan (2012)
Sugarcane	25.0	17.0	12.0	NA	Brown et al. (2012)
Rice straw	32.0	24.0	13.0	47	Karthikeyan and Visvanathan (2012) and Brown (2003)
Eucalyptus	38.0–45.0	12.-13.0	25.0-37.0	NA	Karthikeyan and Visvanathan (2012)
Giant reed stalk	33.1	18.5	24.5	NA	Karthikeyan and Visvanathan (2012) and Ye et al. (2013)
Giant reed leaves	20.9	17.7	25.4	NA	Karthikeyan and Visvanathan (2012)

Sunflower stalk	31.0	15.6	29.2	NA	Monlau et al. (2012a)
Biomass sorghum	22.2	19.4	21.4	NA	Monlau et al. (2012a)
Barley straw	37.5	25.3	26.1	NA	Monlau et al. (2012a)
Rye straw	38.0	36.9	17.6	20	Monlau et al. (2012a)
Napier grass	45.7	33.7	20.6	26	Monlau et al. (2013)
Cornstalk	N.A.*	N.A.	N.A.	27	Monlau et al. (2013) and Nizami et al. (2009)
Oat straw	N.A.	N.A.	N.A.	46	Reddy et al. (2012) and Janejadkarn and Chavalparit (2013)
Cocksfoot grass	N.A.	N.A.	N.A.	12	Wu et al. (2010)
Meadow foxtail grass	N.A.	N.A.	N.A.	14	Wu et al. (2010)
Sorghum stalk	N.A.	N.A.	N.A.	29	Nizami et al. (2009)

*N.A.: Not Available

2.2.3 Anaerobic digestion of crop residues: methane production potential

The anaerobic digestion of cellulosic biomass produces methane gas. The yield of methane gas per unit area is often used to determine the energy productivity of a particular feedstock, and can vary significantly between species, as well as with maturity, geographical location, and inputs (water, fertilizer, etc.) within the same species (Yang et al., 2013). The biochemical methane potential (BMP) test is widely used to examine the anaerobic digestibility of organic substrates. Energy crops, such as switchgrass, miscanthus, and giant reed, have been increasingly studied in recent years as reliable and sustainable biomass feed stocks with high biomass yields and low production costs (Corno et al., 2014). These energy crops can adapt to different climate and soil conditions, and require low fertilizer inputs. Their high water and nitrogen use efficiency enables them to grow on marginal land; thus, they do not compete with food and feed production. About 222,000 ha of marginal land in ten Midwestern U.S. states were identified as suitable for energy crop production (Gelfand et al., 2013; Izaurralde and Zhang, 2013). According to Corno et al. (2014), the biomass yields for switchgrass, miscanthus, and giant reed were about 15, 22, and 45 tons/ha/year, respectively. If 20% of the marginal land was used for growing each of the energy crops, about 3.3 tons of switchgrass, 4.9 tons of miscanthus, and 10.0 tons of giant reed could be produced each year. The methane yield of switchgrass was 113–127 L/kg VS at 35–37°C, and 145–167 L/kg VS at thermophilic condition (Brown et al., 2012; El-Mashad, 2013; Sheets et al., 2015). Miscanthus sinensis harvested in fall and spring showed methane

yields of about 130 and 170 L/kg VS for 30 day and 60 day, respectively (Vasco-Correa and Li, 2015). In addition, reported methane yields by 30-day of giant reed were about 100–147 L/kg VS (Liu et al., 2015a; Yang and Li, 2014). The characteristics of selected energy crops with respect to BMP are summarized in Table 2.4.

Table 2.4 The biomass yield and methane gas production potential of selected cellulosic biomass (Source: Adapted from Weiland, 2010)

Biomass types	Biomass yield (metric ton wet wt./ha)	CH_4 potential (Nm^3 CH_4/metric ton VS)
Sugar beet	40–70	387–408
Fodder beet	80–120	398–424
Maize	40–60	291–338
Wheat	30–50	351–378
Triticale	28–33	319–335
Sorghum	40–80	286–319
Grass	22–31	286–324
Red clover	17-25	297–347
Sunflower	31–42	231–297
Wheat	6–10	371–398

The economic feasibility of anaerobic digestion is strongly contingent on the methane gas potential of the substrate. Higher methane gas production from a given feedstock directly corresponds to shorter payback periods for commercial anaerobic digestion facilities. The feedstock composition is an important factor affecting both the methane yield as well as digester stability; which in turn is governed by the plant species, geographical location, and biomass maturity as discussed previously (Amon et al., 2007b). The authors correlated the effects of harvesting time with biogas production for whole maize (both stover and ear(s)), and found that the best harvesting age with respect to methane yield per hectare was at the end of wax ripeness (i.e., after 122 days). During this stage, the plant contained between 35–39% by wt. (dry basis). At full ripeness (i.e., after 151 days), increases in methane production were minimal. This occurrence can likely be attributed to the carbon to nitrogen (C/N) ratio of the maize, which was much higher than the recommended C/N ratio (i.e., 20–30) for anaerobic digestion (Chandra et al., 2012b). Additionally, the lignin content of the maize may have increased as the crop matured in the field. In general, methane production is known to be less from cellulosic crops which are high in lignin content (Agbor et al., 2011; Alvira et al., 2010). Despite the lower conversion efficiency of the matured crop, however, the highest methane yield per unit area was observed for maize at full ripeness due to the significantly high volatile solids (VS) yield per cropping area. The increased VS content for older maize compensated for a lower methane yield per unit VS added (Schittenhelm, 2008). With respect to other candidate feedstock, like

cereal crops, for example, (e.g., wheat, triticale, and rye) harvesting should be conducted between the grain-in-the-milk stage and grain-in-the-dough stage to obtain the highest methane yield per unit area (Amon et al., 2007a). Similarly, for perennial grasses, the first cut should be conducted after the ear-emergence stage to optimize the methane yield (Amon et al., 2007a).

2.3 Effects of heavy metals on anaerobic digestion [3]

Heavy metals can be present in significant concentrations in municipal sewage and sludge. The heavy metals identified to be of particular concern include cadmium, chromium, cobalt, copper, iron, nickel, and zinc (Jin et al., 1998). A distinguishing feature of heavy metals is that, unlike many other toxic substances, they are not biodegradable and can accumulate to potentially toxic concentrations (Sterritt and Lester, 1980). In one extensive study of anaerobic digester performance, it was found that heavy metal toxicity is one of the major causes of digester upset or failure (Swanwick et al., 1969). The toxic effect of heavy metals is attributed to disruption of enzyme function and structure by binding of the metals with thiol and other groups on protein molecules or by replacing naturally occurring metals in enzyme prosthetic groups (Vallee and Ulner, 1972) (Fig. 2.5).

[3] Significant portions of this part were extracted and rearranged form Chen et al., "Inhibition of anaerobic digestion process: A review", Bioresource Technology, 99 (10), 4044-4064.

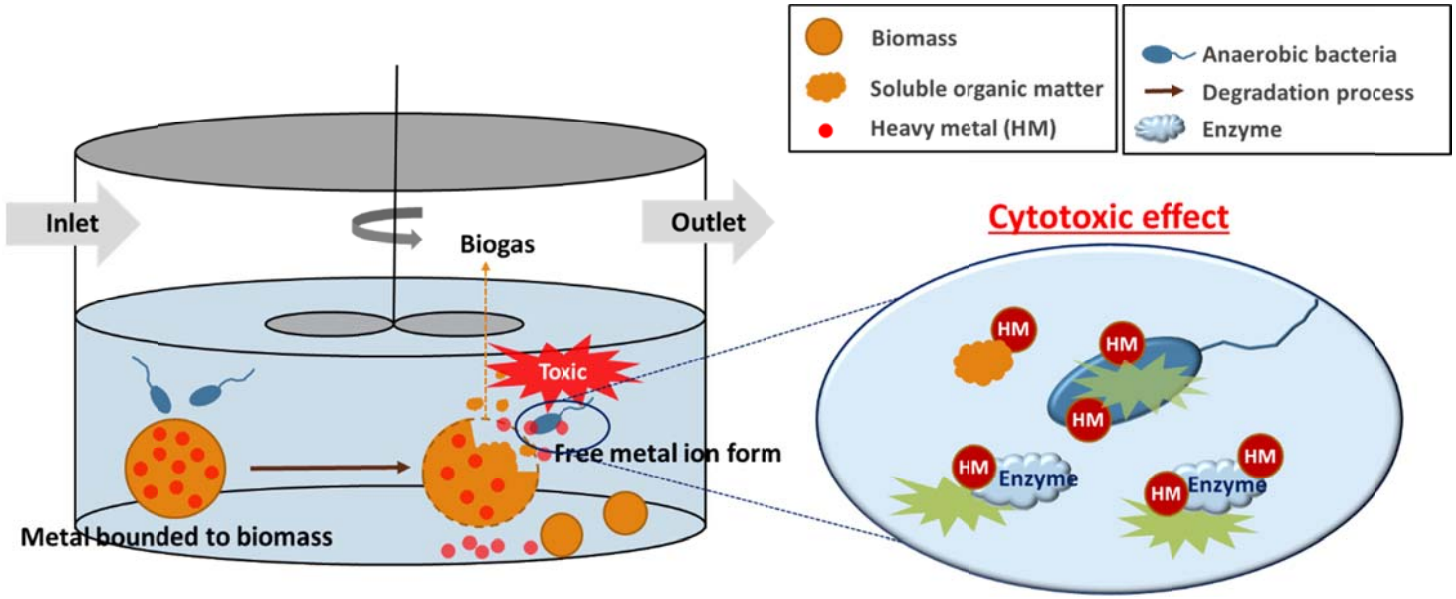

Figure 2.5 Heavy metal effects (i.e., cytotoxic effect) in anaerobic digestion process

2.3.1 Factors of heavy metal inhibition

Many heavy metals are part of the essential enzymes that drive numerous anaerobic reactions. Analysis of ten methanogenic strains showed the following order of heavy metal composition in the cell: Fe ≫ Zn ≈ Ni > Co = Mo > Cu (Takashima and Speece, 1989). Whether heavy metals would be stimulatory or inhibitory to anaerobic microorganisms is determined by the total metal concentration, chemical forms of the metals, and process-related factors such as pH and redox potential (Mosey et al., 1971, Lin and Chen, 1999 and Zayed and Winter, 2000). It is generally believed that acidogens are more resistant to heavy metal toxicity than methanogens (Zayed and Winter, 2000). However, Hickey et al. (1989) have speculated that some trophic group(s) or organisms within the anaerobic consortia in digesters might be more severely inhibited by a pulsed addition of heavy metals than the methanogenic populations.

2.3.2 Chemical forms of heavy metal

Because of the complexity of the anaerobic system, heavy metals may be involved in many physico-chemical processes including (1) precipitation as sulfide (except Cr), carbonate and hydroxides (Lawrence and McCarty, 1965 and Mosey et al., 1971), (2) sorption to the solid fraction, either biomass or inert particulate matter (Shen et al., 1993 and Shin et al., 1997), and (3) formation of complexes in solution with intermediates and product compounds produced during digestion (Hayes and Theis, 1978, Hickey et al., 1989, Callander and Barford, 1983a and Callander and Barford, 1983b). Among these metal forms, only metals in soluble, free form are toxic to the microorganisms (Lawrence and McCarty, 1965, Mosey and Hughes, 1975 and Oleszkiewicz and Sharma, 1990). Several studies have confirmed that the heavy metal toxicity correlated better to the metal's free ionic concentration (determined through a combination of dialysis and ion exchange) than to its total concentration (Bhattacharya and Safferman, 1989, Bhattacharya et al., 1995a and Bhattacharya et al., 1995b). In previous reports, the various physico-chemical forms of a particular heavy metal were rarely distinguished due to the complex interactions between the heavy metals and anaerobic sludge and/or lack of analytical techniques for separating metal species (Gould and Genetelli, 1978, Hayes and Theis, 1978, Oleszkiewicz and Sharma, 1990 and Zayed and Winter, 2000). This is one factor that explains the wide variation in reported toxic concentrations of heavy metals.

2.3.3 Concentrations of heavy metal

In addition to physico-chemical form, differences in substrate, bacteria genre, and environmental factors also explain the wide variation (from several to several hundreds of mg/L) in both the reported dosages of heavy metals and their relative toxicity (Lawrence and McCarty, 1965, Hickey et al., 1989, Bhattacharya et al., 1995a, Jin et al., 1998, Lin and Chen, 1999 and Zayed and Winter, 2000) (Fig. 2.6). Moreover, the operating solids level significantly impacts the heavy metal toxicity in anaerobic digesters by providing protection from metal inhibition. It has been suggested that inhibition due to heavy metals would be more comparable if metal dosage was expressed as mg metal/g VS (Hickey et al., 1989). Unfortunately, most of the literature only reported the inhibition concentration values in mg/L, which makes the comparison of inhibition concentrations more difficult. Heavy metal concentrations that caused 50% inhibition of methanogenesis during whey methanation indicated that toxicity decreased in the order of Cu > Zn > Ni. Similar results were obtained by Lin, 1992, Lin, 1993 and Lin and Chen, 1999.

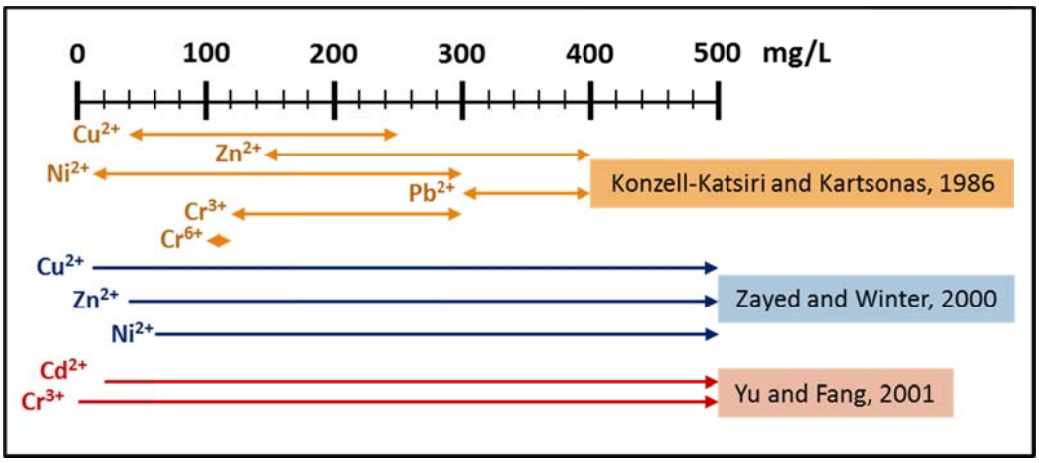

Figure 2.6 Heavy metal toxicity ranges reported in previous studies

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CHAPTER 3
ANAEROBIC DIGESTION AS AN ALTERNATIVE TREATMENT METHOD FOR CROP RESIDUES FROM HEAVY METAL CONTAMINATED SITES

3.1 Introduction

Phytoremediation is an emerging technology as a cost-effective green technology that utilizes plants to clean up contaminated areas (Salt et al., 1995). After the plants are harvested, highly contaminated residues (i.e., plant biomass) must be disposed, and thus, a successful suitable biomass treatment method needs be considered. Contaminated residues are generally disposed of by composting, pyrolysis, direct disposal, incineration, ashing, and anaerobic digestion (Bridgwater et al., 1999; Kumar et al., 1995; Raskin et al., 1997; Sas-Nowosielska et al., 2004).

Among these various treatment methods, the appropriate option should be selected with considerations in terms of the environmental, economical, and energy recovery potential aspects. Composting (Kumar et al., 1995) and pyrolysis (Bridgwater et al., 1999) have a definite advantage in total dry weight reduction of contaminated crop residues. Useful products (i.e., pyrolytic gas) can also be obtained during pyrolysis. However, composting should be post-treated because contaminated biomass will remain after the treatment process (Kumar et al., 1995), thus requiring two to three

months from harvesting to final disposal (Sas-Nowosielska et al., 2004). The main limitations for pyrolysis are its high installation and operation costs. Although direct disposal of contaminated crop residues as hazardous waste is the least complicated approach of disposal, deposition of crop residues at a hazardous waste site is costly (Sas-Nowosielska et al., 2004). In addition, this deposition of crop residues at a hazardous waste site is a reverse trend material recycle movement in waste management field. Although the incineration and ashing processes are advantageous in that they consume less time and allow more biomass reduction relative to other treatment methods (Bridgwater et al., 1999; Raskin et al., 1997), the potential environmental problems are of significant concern. These processes emit large quantities of polluted exhaust gases into atmosphere, and the costs of efficient and adequate gas treatment systems are very high.

Anaerobic digestion also requires large investment, and the overall process is complicated. However, anaerobic digestion may be considered more cost-effective over other methods due to its biomass reduction of crop residues, biogas recovery potential, and low energy consumption during operation (Lehtomäki and Björnsson, 2006). During the anaerobic digestion process, biogas (i.e., methane) converted from volatile compounds can be produced. Although the incineration process also recovers energy from the organic matter in the form of heat (i.e., steam), the main difference between these two processes is that biogas can be stored, while heat energy cannot be stored and converted into other forms of energy. Treatment of crop residues via

anaerobic digestion not only has the advantage of biogas recovery but also offers the benefit of preventing heavy metal emission to the environment by treatment of sludge produced during the anaerobic digestion process. Treatment of sludge can also offer recovery potential of leached valuable metals. Therefore, anaerobic digestion for crop residues harvested from a phytoremediation site should also be considered as a plausible treatment method when considering the environmental, economic, and energy recovery potential aspects.

Heavy metals can exert an important role in anaerobic digestion processes of biomass. Existence of heavy metals can be stimulatory, inhibitory, or even toxic in anaerobic digestion processes depending on their concentrations (Oleszkiewicz and Sharma, 1990). The effects of heavy metals on the anaerobic digestion process have been widely researched over several decades (Bertin et al., 2012; Fang and Chan, 1997). These studies have shown that various factors such as soluble metal concentration (i.e., ionic form in the solution), type of metal species, and amount/distribution of biomass in the digester can cause metal inhibition. This is probably due to the chemical interaction between heavy metals and enzymes of microorganisms, resulting in the disruption of enzyme structure and activities (Li and Fang, 2007). In relatively high concentrations, they can form unspecific compounds and create cytotoxic effects (Kavamura and Esposito, 2010), thus affecting the performance and optimum operating conditions of the processes. Previous researches have reported the inhibitory range of heavy metal concentrations in the anaerobic

digestion process: >20 mg/L for Cd^{2+} (Yu and Fang, 2001), >1 mg/L for Cu^{2+}, >10 mg/L for Ni^{2+}, >4 mg/L for Zn^{2+} (Kouzeli-Katsiri and Kartsonas, 1986; Yenigün et al., 1996; Zayed and Winter, 2000), and >30 mg/L for Pb^{2+} (Kouzeli-Katsiri and Kartsonas, 1986). For successfully applying anaerobic digestion to treatment of contaminated crop residues, the effects of heavy metals-containing biomass on anaerobic digestion has to be considered.

The aim of this research was to investigate the applicability of anaerobic digestion as a treatment method of crop residues harvested from heavy metal contaminated site. If there were no significant effects of the high heavy metals-containing crop residues on the anaerobic digestion process, anaerobic digestion could be suggested as a treatment method of crop residues cultivated in heavy metal phytoremediation sites. Biochemical methane potential (BMP) test was conducted using sunflowers (i.e., *Helianthus annuus*) collected from various concentrations of heavy metals contaminated soils as crop residues. Methane gas production was considered an indicator for monitoring an anaerobic digestion process suffering from endogenous heavy metals. The results of the test were compared to the reported methane gas production of various grass crop residues from prevalent farmland for evaluating energy recovery potential.

3.2 Materials and methods

3.2.1 Preparation and characterization of substrate

In this study, sunflower containing heavy metals from a phytoremediation site was used as crop residues. Since the sunflower has high heavy metal accumulating capacity in its biomass and good tolerance to various heavy metals (Lee et al., 2013), it is the most frequently used plant species for the remediation of heavy metal contaminated sites. In addition, the sunflower has relatively high biomass production compared to other plants and could be easily cultivated in various soil textures in Korea. Four sunflowers grown in different heavy metals contaminated soils (i.e., field contaminated soil, two differential concentrations of artificially contaminated soils, farmland soil as a control) were used in this study. The heavy metal concentrations in four types of soils are shown in Table 3.1.

Table 3.1 Heavy metal concentrations in soils for cultivation of sunflowers

Unit: mg heavy metal/kg soil (dry basis)

	Cd	Cu	Ni	Pb	Zn
Sunflower I (Field soil _ abandoned mine)	3.98	29.17	28.65	155.13	236.25
Sunflower II (Artificially contaminated soil _ moderate conc.)	2.67	17.40	3.67	105.66	145.81
Sunflower III (Artificially contaminated soil _ maximum conc.)	30.49	42.93	7.29	225.59	229.72
Sunflower IV (Control _ purchased from market)	N.A.*	N.A.	N.A.	N.A.	N.A.

*N.A.

‘Sunflower I’ was grown for 120 days in a heavy metal contaminated site near abandoned mine at Jecheon-si, Chungcheongbuk-do, Korea. Physicochemical properties of soils from Jecheon showed organic matter content of 2.51% by dry wt., pH of 6.9, and texture of silt loam (sand 27, silt 55.6, and clay 17.4% by dry wt.). ‘Sunflower II and III’ were grown for 100 days in a greenhouse for two different concentrations of artificially contaminated soils. They were cultivated in artificially heavy metal contaminated soils. The soil was collected from farmland at Hapcheon-gun, Gyeongsangnam-do, Korea. The physicochemical properties of the soils from Hapcheon were organic matter content of 3% by dry wt., pH of 6.5, and texture of sandy loam (sand 71.1, silt 15.9, and clay 13.0% by dry wt.). After the soil was collected, two levels of heavy metals (i.e., Cd, Pb, Ni, Cu, and Zn) were spiked into the soil for ‘Sunflower II and III’ in this paper. Heavy metal concentration in soil of ‘Sunflower II’ is a moderate level of heavy metal concentrations in soils to generally conduct phytoremediation, thus the crop residues grown in these soils contain relatively low levels of heavy metals. Heavy metal concentration in soil for ‘Sunflower III’ is the maximum level of heavy metals in soils for normal growth of sunflower obtained from our previous research, indicating that crop residues harvested from these soils contain the highest concentrations of heavy metals in biomass. Sunflowers cultivated in prevalent farmland were used as the control in this study and were obtained from a commercial market. All parts of the sunflowers (i.e., stems, leave, and flowers) were mixed and used in a BMP test. The seeds, which can be used as source

of biodiesel, were not used in this study.

Proximate analysis was carried out according to the American Society for Testing and Materials (ASTM) standard test method E871-82, E872-82, and E1755-01 (ASTM, 2006a, 2006b and 2007). The elemental composition (e.g., carbon, hydrogen, oxygen, nitrogen, and sulfur contents) of crop residues was determined using an elemental analyzer (Flash2000, Thermo, USA). For elemental composition analysis, the crop residues were completely oven-dried at 70°C and oven-dried crop residues were ground into fine powder.

For heavy metal analysis, the crop residues were oven-dried to remove moisture completely until constant weight of crop residues was maintained. Oven-dried crop residues were ground into fine powder and then digested with a solution of HNO_3, H_2O_2, and distilled H_2O (9:1:1, v/v/v) using a microwave digester (MSP1000, CEM, USA), according to the US Environmental Protection Agency (US EPA) 3052 method for heavy metal analysis (US EPA, 1996). After digestion, the volume of each sample was adjusted to 25 mL with distilled water. The concentrations of heavy metals in crop residues were determined by ICP-OES (iCAP 7400, Thermo, USA).

For structural analyses, the fibre composition of crop residues is routinely determined using the neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid digestible lignin (ADL). The analyses were performed according to the Association of Official Analytical Chemists (AOAC) official method 973.18 and 2002.04 (AOAC, 2005). The hemicellulose and cellulose contents were calculated

from the obtained NDF, ADF, and ADL. The hemicellulose and cellulose contents were calculated as the difference between NDF and ADF, and ADF and ADL, respectively. Lignin content was determined gravimetrically from ADL as the residue remaining upon ignition after 72% H_2SO_4 treatment.

3.2.2 General methods of BMP test

BMP test was conducted as a tool for evaluating the anaerobic digestion process. The BMP test can be used as an index of the anaerobic biodegradation potential as it is the experimental value of the maximum quantity of methane produced per gram of VS.

The BMP tests carried out in this study followed and modified the procedure as described by Owen et al. (1979) in 250 mL serum bottles (Fig. 3.1). The effective liquid volume in each bottle was 100 mL for the experiments. Each serum bottle contained an organic loading of 0.5 g VS/L of crop residues. The nutrient and trace metal solution for the optimal function of the anaerobic microorganisms was prepared using the method described by Shelton and Tiedje (1984) and added at 90% (v/v) of the total inoculated medium. Serum bottles were seeded with anaerobic sludge obtained from Jungnang Sewage Treatment Plant, and the amount was 10% (v/v) of the total inoculated medium. The assay bottles were flushed continuously with N_2 gas for five minutes to make anaerobic conditions, after which they were sealed with a butyl rubber stopper and capped with aluminum crimp. A constant internal temperature of 35±1°C and 150 rpm was achieved in a temperature controlled mechanical shaker. All bottles

were set in triplicate.

Gas production and gas composition were analyzed to assess the efficiency of the anaerobic digestion on each BMP test by t-valve gas flow meter and gas chromatography everyday (ACME 6100, Younglin, Korea). Gas chromatography was operated with a thermal conductivity detector (TCD) at 120℃, with injector and oven temperatures at 120 and 35°C, respectively. Helium was used as the carrier gas.

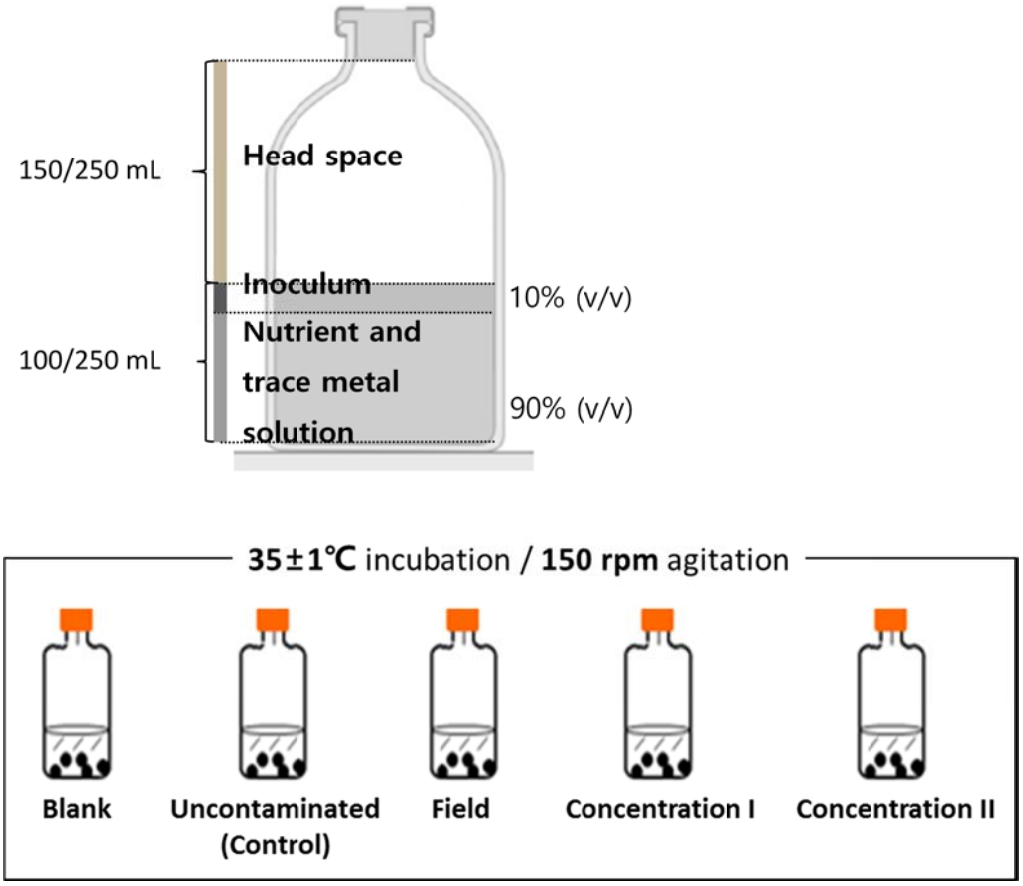


Figure 3.1 Design of BMP test in this study

3.2.3 Distribution of heavy metals after BMP test

After the BMP test, all bottles were opened and the mixtures in the bottles were centrifuged for 15 minutes at 15,000 rpm to investigate the distribution of heavy metals in each liquid and solid phase. The heavy metal concentrations dissolved in liquid phase were measured from supernatants passing through a glass fiber filter (0.45 μm nominal pore size). The solid phase was dried at 105°C overnight and ground into a fine powder, and the heavy metals in the solid phase were determined according to the US EPA 3052 method (US EPA, 1996). The heavy metal concentrations in each phase were analyzed using ICP-OES (iCAP 7400, Thermo, USA).

3.3 Results and discussion

3.3.1 Characterization of substrate

The physicochemical properties of the crop residue from proximate, elemental, and structural analysis and heavy metal concentration in crop residues used in this study (i.e., sunflower) is shown in Table 3.2. The moisture content and volatile solids (VS, % of total solids) of crop residues were observed at 59.65±1.01% by wt. (wet basis) and 83.08±1.01% by wt. (dry basis), respectively. According to the structural analysis, the crop residues were composed of 11.5±0.61, 62.75±1.84, and 25.75±1.26% of lignin, cellulose, and hemicellulose, respectively. Elemental composition analysis showed 42.05±0.37 of carbon and 2.19±0.15 of nitrogen % by wt. (dry basis), and the carbon to nitrogen (C/N) ratio was calculated as 19.28±1.13. From the elemental composition analysis, the theoretical methane production potential of crop residues used in this study was calculated as 499.21±4.80 mL/g VS using the following equation (Eq. 3.1) suggested by Rich (1963):

$$C_aH_bO_cN_d + \left[\frac{4a-b-2c+3d}{4}\right]H_2O \rightarrow \left[\frac{4a+b-2c-3d}{8}\right]CH_4 + \left[\frac{4a-b+2c+3d}{8}\right]CO_2 + dNH_3 \qquad \text{Eq. 3.1}$$

The C/N ratio of substrate is an important parameter in the anaerobic digestion process, and a proper C/N ratio value is necessary for process optimization. The range of optimal C/N ratio varies with the type of substrate to be digested. Generally, the

optimum C/N ratio for anaerobic digestion is agreed to be in the range of 20-30 (Li et al., 2011). Wu et al., (2010) obtained the highest average biogas volume for oat straw at C/N ratio of 20. A low C/N ratio could lead to accumulation of potential inhibitors such as total ammonia-N (TAN) and volatile fatty acid (VFA) (Li et al., 2011), whereas the rapid consumption of nitrogen and low biogas production could be caused by high C/N ratio (Kayhanian, 1999). The C/N ratio of crop residues used in this study was included in appropriate range of C/N ratio for anaerobic digestion. Therefore, anaerobic digestion could be considered an appropriate treatment method for crop residues used in this study.

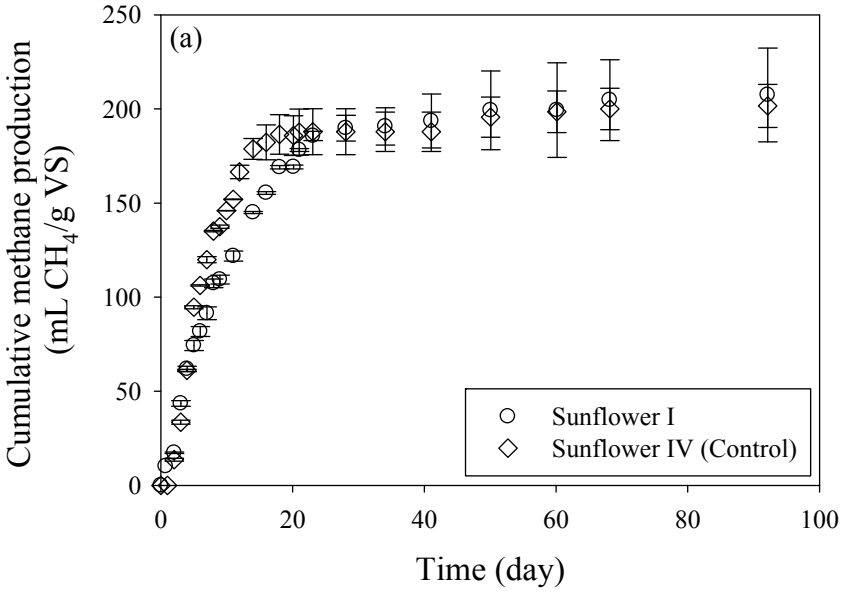
Table 3.2 Characterization of crop residues used in this study

			Sunflower I	Sunflower II	Sunflower III	Sunflower IV (Control)
Proximate analysis	% by wt. (wet basis)	Moisture	60.0	58.3	59.6	60.7
		TS	40.0	41.7	40.4	39.3
	% by wt. (dry basis)	VS	84.0	82.3	82.1	83.9
		FS	16.0	17.7	17.9	16.1
Elemental analysis	% by wt. (dry basis)	C	41.9	41.6	42.4	42.3
		H	5.40	5.20	4.90	5.44
		N	2.06	2.11	2.19	2.39
		O	42.9	43.1	42.2	46.1
		S	0.22	0.21	0.20	0.26
		Ash	7.52	7.78	8.11	3.51
Structural analysis	% by wt. (dry basis)	Lignin	10.7	12.1	11.8	11.4
		Cellulose	65.0	61.2	61.3	63.5
		Hemicellulose	24.3	26.7	26.9	25.1

Heavy metal concentration	mg/kg crop residue (dry basis)	Cd	3.21	4.45	58.4	2.82
		Cu	26.3	20.1	23.0	1.41
		Ni	1.45	0.41	2.01	0.21
		Pb	13.1	3.43	9.88	8.86
		Zn	56.0	67.9	146	51.6

3.3.2 Effect of heavy metal concentrations in crop residues on anaerobic digestion

The cumulative methane productions of crop residues from BMP tests are shown in Fig. 3.2. Maximum methane production was observed at 201.60±11.39 and 207.42±34.90 mL/g VS (n=3) from 'Sunflower IV' and 'Sunflower I', respectively (Fig. 3.2 (a)). A significant difference in cumulative methane production was not observed. Fig. 3.2 (b) shows 227.38±15.59 and 217.21±6.07 mL/g VS (n=3) of cumulative methane production in crop residues harvested from 'Sunflower II' and 'Sunflower III', respectively. Although the crop residues were grown under the maximum level of heavy metal for normal growth of sunflowers (i.e., Sunflower III), there was also no significant difference with Control in terms of cumulative methane production. The only difference between four crop residues harvested from differential soils was the time to reach their own maximum methane production. The results of BMP tests suggest that there were no adverse effects of heavy metals from crop residues on anaerobic bacterial activity and methane production was not hindered. Due to the above results and the differences between heavy metal amounts within each crop residue, it may be conjectured that all four crop residues used in this study were affected by a similar amount of heavy metal, which is in a form that could inhibit bacterial activity.



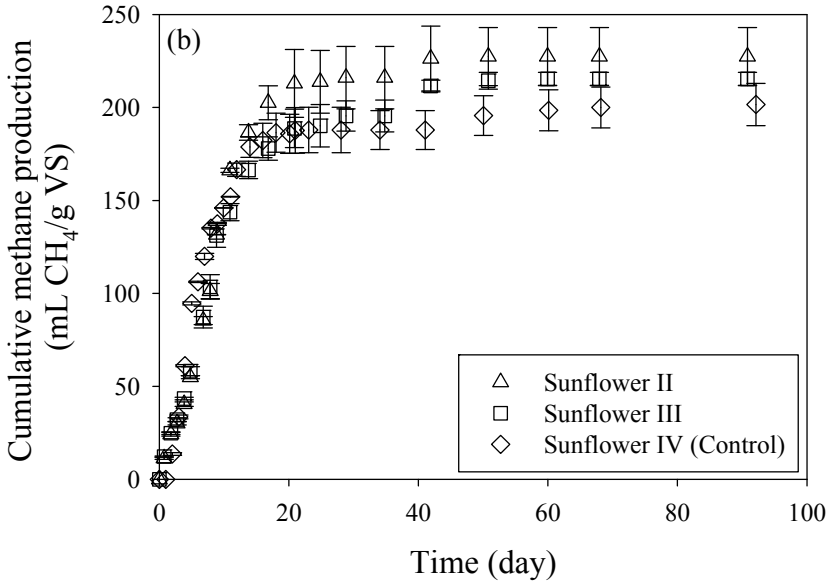


Figure 3.2 Cumulative methane production from crop residues harvested from differential amounts of heavy metal-containing soils: Comparison between (a) Sunflower I and Control, (b) Sunflower II, Sunflower III, and Control

In order to investigate the distribution of heavy metals in crop residues, the heavy metals in crop residues after the BMP test were classified into two phases: solid phase (i.e., biomass) and liquid phase (i.e., solution) (Fig. 3.3). Heavy metals, which are attributed to seeding sludge, were calculated from the result of the blank test and were excluded. Distribution of Cu and Zn were observed in mixture for four types of crop residues, shown in Fig. 3.3 (a) and (b), respectively. The concentrations of heavy metals in the liquid phase, which are known to be directly affecting anaerobic bacterial activity, were similar. Whereas heavy metals that exist in the solid phase cannot directly affect the anaerobic process efficiency, heavy metals existing in the liquid phase can inhibit bacterial activity when the amount is greater than the inhibitory level (Lawrence and McCarty, 1965). Heavy metal inhibition depends upon the type of metal species and concentrations of heavy metals that are present in soluble form. Oleszkiewicz and Sharma (1990) demonstrated that only soluble forms can be considered to predict the inhibitory response of heavy metals in an anaerobic digester. Bhattacharya et al. (1995) also concluded that heavy metal toxicity can be strongly dependent upon the free ionic concentration of the metal in solution rather than the total metal concentration. The soluble concentration of heavy metals needs to be monitored carefully due to its toxicity to bacteria under anaerobic conditions. Moreover, the amounts of heavy metals from crop residues in the liquid phase were below the inhibitory levels of Cu and Zn, which are reported to be toxic to anaerobic bacteria (i.e., cytotoxic effect) (Kouzeli-Katsiri and Kartsonas, 1986; Yenigün et al.,

1996; Zayed and Winter, 2000). Although the wide variation in the reported dosage of heavy metals for inhibition can depend on differences in substrate, adaption of bacteria, and anaerobic digester operating conditions (Bhattacharya et al., 1995; Lawrence and McCarty, 1965; Zayed and Winter, 2000), some literatures have reported ranges of heavy metals inhibitory to the anaerobic digestion process. Zayed and Winter (2000) found that anaerobic bacteria can be inhibited at the concentrations of over 1 mg/100 mL for copper and over 4 mg/100 mL for zinc. Yenigün et al. (1996) reported that copper ion inhibits the anaerobic digestion process within the concentration range of 0.1-1 mg/100 mL, and zinc ion is inhibitory to anaerobic digestion process at a concentration range of 0.5-4 mg/100 mL.

The above results may also be explained with the maximum theoretical heavy metals amounts released from crop residues, which can be derived from the organic loading rate of crop residues, VS contents of crop residues, and heavy metal concentrations in crop residues. The theoretically calculated maximum amounts of heavy metals released from crop residues in 100 mL of liquid phase were 0.001-0.016 mg of Cu and 0.031-0.087 mg of Zn, respectively. Thus, the theoretical maximum heavy metals amounts released from crop residues, being less than the reported inhibitory ranges of heavy metals, can also support the finding that there were no adverse effects of heavy metals in crop residues onto anaerobic bacterial activities and no significant differences in methane productions.

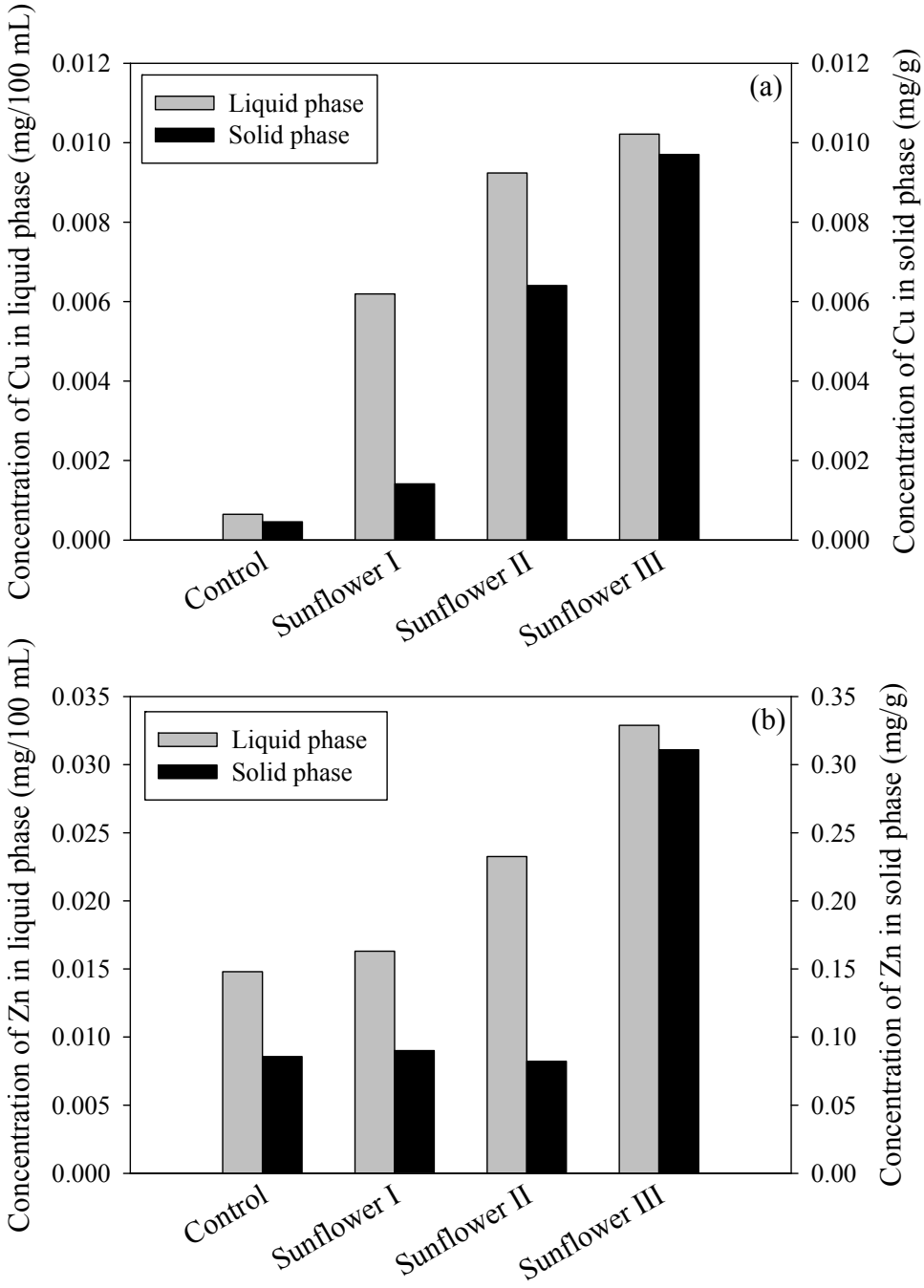


Figure 3.3 Distribution of Cu (a) and Zn (b) in mixture after BMP test

3.3.3 Evaluation of energy recovery potential for anaerobic digestion using crop residues

Heavy metal concentrations in crop residues were confirmed to be below the concentration range of adverse effects. As it has been confirmed that crop residues cultivated on phytoremediation site could be disposed by anaerobic digestion, energy recovery potential was compared to the reported methane gas production of other grass crop residues cultivated on prevalent farmland (Table 3.3).

Table 3.3 Methane gas production potential from previous studies

Plant	Fermenter	Temp. (℃)	CH_4 yield (L/g VS)	References
Sunflower	BMP assay	35	0.20-0.23	This study
			0.19-0.24	Antonopoulou et al., 2010
				Monlau et al., 2012
	N.A.*	N.A.*	0.23-0.30	Deublein et al., 2010
Energycane	BMP assay	35	0.24-0.32	Chynoweth et al., 2001
Napiergrass	BMP assay	35	0.19-0.34	Chynoweth et al., 2001
				Shiralipour et al., 1984
				Tong et al., 1990
Sorghum	BMP assay	35	0.28-0.38	Chynoweth et al., 2001
				Richards et al., 1991
Corn stover	BMP assay	35	0.36	Tong et al., 1990

*N.A.: Not Reported

Methane gas production of sunflowers from prevalent farmland were reported to be about 190 and 240 mL/g VS from BMP tests provided by Monlau et al. (2012) and Antonopoulou et al. (2010), respectively. Deublein and Steinhauser (2011) reported the methane gas production of sunflower as 230-300 mL/g VS. These values are quite similar to the maximum methane gas production of crop residues used in this study (i.e., sunflower). The methane gas production of other grass crop residues via BMP test were reported to range from 190 to 380 mL/g VS. Grass crop residues generally consist of cellulose, hemicellulose, and lignin, which accounts for 30-50 (can make up as much as 61), 25-30, and 10-20% by wt. of dry biomass, respectively (Smil, 1999). The compositions of sunflowers used in this study were 62.75±1.84, 25.75±1.26, and 11.5±0.61% by wt. (dry basis) of cellulose, hemicellulose, and lignin, respectively. This difference in cellulose contents could have an effect on methane gas production. Although the methane gas production of other crop residues was relatively higher than that of sunflowers, it could be comparable. Therefore, sunflower residues harvested from heavy metal contaminated soil could be disposed by anaerobic digestion, and could be considered as a substrate for biogas production.

When crop residues used in this study (i.e., sunflowers harvested from heavy metal phytoremediation site) are disposed by anaerobic digestion, the generation of electricity was estimated based on the methane gas production potential from the BMP test results of field soil (Table 3.4). The calorific value of methane gas was reported to be about 35,600 kJ/m^3, as provided by Wiley et al. (2011). Considering 213.73 mL/g

VS of methane production, 60% by wt. (wet basis) of moisture content of sunflower residue, and 84% by wt. (dry basis) of volatile solid to total solid ratio of sunflower residue, 71,844 L of CH_4 could be generated from one ton of sunflower residue. The calorific value of CH4 from sunflower residue was calculated to be 2,557,646 kJ/ton. 213.14 kWh of electricity could be produced from a ton of sunflower residue harvested from a phytoremediation site, assuming that the efficiency of electricity generation is approximately 30% as provided by Sallaku et al. (2010).

Table 3.4 Estimation of electricity generation potential of sunflower residues from a heavy metal contaminated site with anaerobic digestion

Parameter	Estimated value
Calorific value of CH_4	35,600 kJ/m^3
Moisture content of sunflower residue	60% by wt. (wet basis)
VS/TS of sunflower residue	84% by wt. (dry basis)
Maximum CH_4 production of sunflower residue	207.42 mL/g VS
Biochemical CH_4 potential of sunflower residue	213.73 mL/g VS (= 71,844 L/ton, wet basis)
Calorific value of CH_4 from sunflower residue	2,557,646 kJ/ton (= 710.46 kWh/ton)
Efficiency of electricity generation	30%
Electricity generated from sunflower residue	213.14 kWh/ton

3.4 Summary

Despite the fact that the crop residues may be an attractive substrate for methane production during anaerobic digestion, this may be an inappropriate consideration in the case of harvesting crop residues from heavy metal contaminated sites due to endogenous heavy metals in crop residues. Existence of heavy metals in the anaerobic digestion process may cause adverse effects, resulting in toxicity to bacterial activity and causing possible process failure. In this study, although the crop residues contained differential amounts of heavy metals including the maximum level of heavy metal for normal growth of sunflower, there was no significant difference in methane gas production between crop residues. This is due to the amount of heavy metals that are in the form (i.e., liquid phase) in which directly affects anaerobic bacterial activity. In sum, the anaerobic bacterial activity for methane gas production was unhindered by the existence of endogenous heavy metals in crop residues. The results of this study revealed that anaerobic digestion could be a plausible alternative treatment method in terms of energy recovery potential for crop residues from heavy metal contaminated sites.

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CHAPTER 4
STABILITY OF ANAEROBIC DIGESTION FOR CROP RESIDUES FROM HEAVY METAL CONTAMINATED SITES WITH LAB-SCALE CSTR

4.1 Introduction

Anaerobic digestion is a biological process in which microorganisms degrade organic matter and convert it into biogas as the end product. Agricultural crop residues represent an important source of biomass that can be utilized as a substrate in anaerobic digestion (Prabhudessai et al., 2013). Anaerobic digestion for crop residues has been applied as an effective technology in terms of renewable energy production, byproduct utilization, and agricultural waste reduction (Zhang et al., 2013). Cuetos et al. (2011) conducted semi-continuous reactor to determine the methane yields for crop residues, and the results were 0.30±0.01, 0.34±0.03, and 0.26±0.02 L CH_4/g VS for maize, rapeseed, and sunflower residues, respectively. In addition, methane production of crop residues has been found to range between 0.19±0.01 to 0.31±0.01 L CH_4/g VS for leaves of cauliflower (*Brassica oleracea* var. botrytis) and cabbage (*Brassica oleracea* var. capitata), respectively (Gunaseelan, 2004). It has been proposed that crop residues harvested in heavy metal contaminated sites may have the potential advantage of becoming economically and environmentally attractive in terms of efficient land

utilization and soil remediation (Evangelou et al., 2012). Studies on anaerobic digestion have been only focused on the energy crop residues (e.g., clover, wheat straw, corn stalks, and rice straw) as the substrates to produce biogas through anaerobic digestion (Agneessens et al., 2014), and the application of anaerobic digestion for crop residues containing heavy metals from phytoremediation sites has not been investigated.

Although anaerobic digestion may be an attractive treatment method for crop residues, it may be an improper consideration in the case of cultivating crop residues from heavy metal phytoremediation sites due to the endogenous heavy metals in crop residues. The existence of heavy metals in the anaerobic digestion process may cause adverse effects, resulting in toxicity to microbial activities and causing possible process upset or failure. The adverse effect (i.e., toxicity) of heavy metals is attributed to the interruption of enzyme function and structure by the forming of metal complex with thiol and other groups on protein molecules or by replacing naturally occurring metals in enzyme prosthetic groups (Vallee and Ulmer, 1972). Previous studies have reported that various factors such as soluble metal concentration (i.e., ionic form in the solution), type of metal species, and amount/distribution of biomass in the digester can cause metal inhibition (Bertin et al., 2012; Fang and Chan, 1997). Among various factors, existing forms of heavy metals and concentrations of soluble heavy metals are known to be significant factors. Previous studies have confirmed the inhibitory ranges of heavy metal concentrations in anaerobic digestion process to be >20 mg/L for Cd^{2+} (Yu

and Fang, 2001), >1 mg/L for Cu^{2+}, >10 mg/L for Ni^{2+}, >4 mg/L for Zn^{2+} (Kouzeli-Katsiri and Kartsonas, 1986; Yenigün et al., 1996; Zayed and Winter, 2000), and >30 mg/L for Pb^{2+} (Kouzeli-Katsiri and Kartsonas, 1986). Most studies are exclusively focused on the inhibition of soluble heavy metal to anaerobic digestion and there are a few studies on the effects of endogenous heavy metals within substrate. The effects of heavy metals in crop residues on anaerobic digestion process should be studied to secure sustainable application of anaerobic digestion for heavy metal-containing crop residues.

The objective of this research was to investigate the long-term stability on the performance of anaerobic digestion for the treatment of crop residues harvested in heavy metal contaminated sites. To achieve this goal, a laboratory-scale reactor was operated under anaerobic condition with sunflower harvested from heavy metal contaminated site. The effects of endogenous heavy metals on the reactor performance were investigated. Additionally, to investigate the heavy metal effects on the structure and diversity of bacterial and archaeal communities, the microbial communities was identified by using pyrosequencing.

4.2 Materials and methods

4.2.1 Substrate and inoculum

In this study, sunflower (i.e., *Helianthus annuus*) containing heavy metals from a phytoremediation site was used as the substrate. Sunflower is the most frequently used biomass for remediation of heavy metal contaminated site due to its high heavy metal accumulating capacity (Lee et al., 2013; Lone et al., 2008) and its relatively high biomass production compared to other plants (Zhuang et al., 2005). In addition, sunflower can easily be cultivated in various soil textures of the Republic of Korea. The sunflower used in this study was grown for 120 days in a heavy metal contaminated site near abandoned mine at Jecheon-si, Chungcheongbuk-do, Republic of Korea. This site was contaminated with 3.98 mg-Cd, 29.17 mg-Cu, 28.65 mg-Ni, 155.13 mg-Pb, and 236.25 mg-Zn/kg-soil, respectively. Physicochemical properties of soils in site showed organic matter content of 2.51% by dry wt., pH of 6.9, and texture of silt loam (sand 27, silt 55.6, and clay 17.4% by dry wt.). Since the sunflower used in this study was cultivated from aforementioned heavy metal contaminated site, the sunflower contained 3.21 mg-Cd, 26.3 mg-Cu, 1.45 mg-Ni, 13.1 mg-Pb and 56.0 mg-Zn/kg sunflower, respectively. All parts of harvested sunflowers (i.e., stem, leaf, and flower) were ground into fine particles using a blender. Ground substrate was mixed in order to apply homogeneity and to facilitate injection.

Sewage sludge was obtained from a waste water treatment plant in Seoul,

Republic of Korea and used as the inoculum. Acquired inoculum was pretreated using a sieve with a pore size of 500 μm for the purpose of removing impurities. No additional alkalinity, or buffer, was introduced into the inoculum. The characterizations of sunflower and seeding sludge are summarized in Table 4.1.

Table 4.1 Characterization of substrate and inoculum used in this study

	Sunflower (Substrate)	Seeding sludge (Inoculum)	Unit
Moisture	60.0	96.4	% by wt. (wet basis)
VS	33.6	2.19	
FS	6.40	1.41	
C	41.9	25.2	% by wt. (dry basis)
H	5.40	3.83	
N	2.06	3.06	
O	42.9	17.8	
S	0.22	1.11	
Ash	7.52	49.0	
Cellulose	65.0	N.A.	% by wt. (dry basis)
Hemicellulose	24.3	N.A.	
Lignin	10.7	N.A.	

N.A. Not analyzed

4.2.2 CSTR operation

A continuous stirred-tank reactor (CSTR) with a working volume of 5 L (total volume of 8 L) was used in this study (Fig. 4.1). Anaerobic condition was achieved by purging the reactor with nitrogen gas before the digestion process and the reactor was operated under mesophilic condition at 35±1°C. Consistent stirring was managed using an electrical motor attached to the reactor. The substrate was fed once a day with pulse feeding method using 50 mL plastic syringe. The initial organic loading rate (OLR) of reactor was set as 0.5 g VS of sunflower/L/day daily feeding rate for three months to acclimate with the sunflower substrate. After three months of acclimation period, the OLR was increased stepwise from 1.0 to 2.0 g VS of sunflower/L/day (Table 4.2).

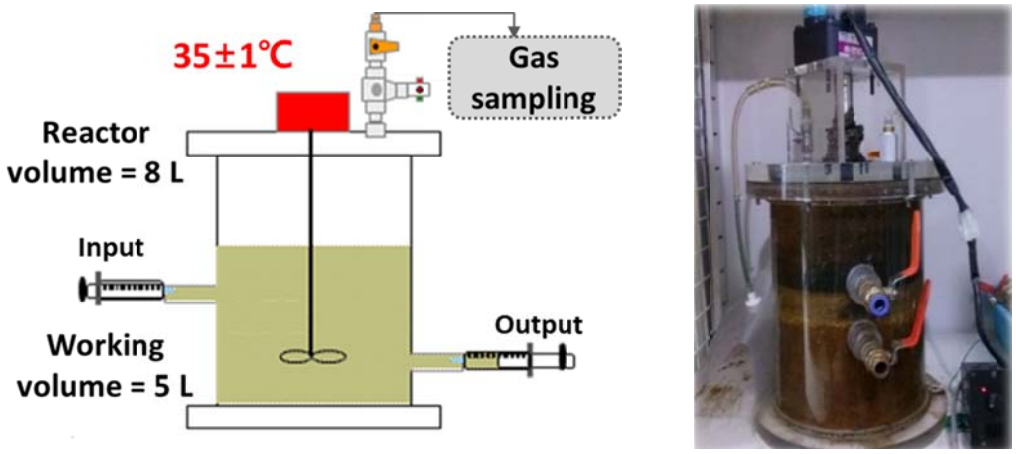


Figure 4.1 Schematic design of lab-scale CSTR used in this study

Table 4.2 Summary of reactor operating condition

	Phase I	Phase II	Phase III-1	Phase III-2	Phase III-3
HRT (days)	30	24	20	20	20
OLR (g VS/L/day)	1.0	1.25	1.5	1.75	2.0
Operating period (days)	103	175	622	100	100

In order to allow acclimation period for the anaerobic microorganisms within reactor, gradual and careful changes in the environment was employed. In order to investigate the effects of heavy metals on anaerobic digestion, OLR was gradually increased. When heavy metal-containing substrate is fed to the reactor, changes in OLR and HRT can indicate changes in absolute heavy metal concentrations within the reactor. The anaerobic reactor operation can be summarized into three different phases: Phase I, II, and III. Phase I had a hydraulic retention time (HRT) of 30 days with an OLR of 1.0 g VS/L/day for 103 days, and phase II had an HRT of 24 days with an OLR of 1.25 g VS/L/day for the next 175 days. Phase III had a constant HRT of 20 days, but the OLR was sequentially increased from 1.5 (Phase III-1) to 1.75 (Phase III-2) and 2.0 g VS/L/day (Phase III-3). Phase III-1 had operated for 622 days, and the remaining

phases (Phases III-2 and 3) had operated for 100 days each. The raised OLR up to 2.0 g-VS/L/day can be considered relatively high as the reported OLR for anaerobic digestion of crop residues ranges from 1.3 to 2.3 g VS/L/day (Stewart et al., 1984; Wilkie et al., 1986).

4.2.3 Analytical methods

For heavy metal analysis, the leachate was oven-dried to remove moisture completely until constant weight of leachate was maintained. Oven-dried leachate was ground into fine powder and then digested with a solution of HNO_3, H_2O_2, and distilled water (9:1:1, v/v/v) using a microwave digester (MSP1000, CEM, USA) according to the US Environmental Protection Agency (US EPA) 3052 method for heavy metal analysis. After digestion, the volume of each sample was adjusted to 25 mL with distilled water. The concentrations of heavy metals in crop residues were determined by ICP-OES (iCAP 7400, Thermo, USA).

Several parameters have been commonly suggested as indicators for anaerobic digestion stability including biogas production, methane content in biogas, volatile fatty acids (VFAs) concentrations, alkalinity, organic matter decomposition, and pH value (Ahring et al., 1995). Biogas (i.e., methane, carbon dioxide, and nitrogen) production was measured with a wet-type gas flow meter. Each recorded biogas volume was converted into standard temperature and pressure conditions (273 K, 101.325 kPa). Methane content in the biogas was measured by gas chromatography

(ACME 6100, Younglin, Korea) with a thermal conductivity detector (TCD), injector, and oven operating at 120, 120, and 35°C, respectively. Helium was used as the carrier gas.

VFAs concentrations were also measured using a gas chromatography (ACME 6100, Younglin, Korea) with a flame ionization detector (FID). The main VFAs of interest were acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, and hexanoic acid. The injector and FID temperatures were 240 and 250°C, respectively. The initial oven temperature was 100°C for 2 min, with a 10°C/min ramp up to 190°C.

Alkalinity (as $CaCO_3$) and chemical oxygen demand (COD) of the effluent were monitored. Alkalinity was analyzed according to standard methods (APHA, 2005) and COD was measured using water quality analyzing kit product (Water Test Kit, Humas, Korea). The product is based on the AWWA standard test method. For soluble COD (SCOD) measurement, the soluble portion of effluent was obtained from filtrate passing through a glass fiber filter (0.45 μm nominal pore size).

4.2.4 Microbial community analysis: DNA extraction, PCR, and pyrosequencing

Bulk genomic DNA was extracted from 0.5 mL of raw sludge and leachate of CSTR at every OLR increasing point, using a FastDNA SPIN Kit for Soil (MP Biomedicals, USA). The extracted DNA was amplified, using primers targeting the V1 to V3 regions of 16S rRNA gene. The primer sequences were as following: bacteria-

specific primers 9F (5'-CCTATCCCCTGTGTGCCTTGGCAGTC-TCAG-AC-AGAGTTTGATCMTGGCTCAG-3') and 541R (5'-CCATCTCATCCCTGCGTGTCTCCGAC-TCAG-X-AC-ATTACCGCGGCTGCTGG-3'); archaea-specific primers 338F (5'- CCTATCCCCTGTGTGCCTTGGCAGTC-TCAG-AG-CAGCCGCCGCGGTAA-3') and 926R (5'-CCATCTCATCCCTGCGTGTCTCCGAC-TCAG-X-GA-YCCGGCGTTGAMTCCAATT-121-3'). 'X' indicates the unique barcode for each subject (http://oklbb.ezbiocloud.net/content/1001).

Amplifications were carried with DNA denaturation, at 95°C for 5 min, followed by 30 cycles at 95°C for 30 sec, primer annealing at 55°C for 30 sec, extension at 72°C for 30 sec, and a final elongation at 72°C for 5 min. The PCR product was confirmed by using 2% agarose gel electrophoresis and visualized under a Gel Doc system (BioRad, Hercules, CA, USA). The sequencing was carried out at Chunlab, Inc. (Seoul, Korea), with GS Junior Sequencing system (Roche, Branford, CT, USA) according to the manufacturer's instructions.

For the taxonomic assignment of each pyrosequencing read, the EzTaxone database, which contains 16S rRNA gene sequences of type strains that have valid published names and representative species-level phylotypes of either cultured or uncultured entries in the database, was used. Individual sequence reads were taxonomically assigned according to the following criteria (x = similarity): species ($x \geq 97\%$), genus ($97 > x \geq 94\%$), family ($94 > x \geq 90\%$), order ($90 > x \geq 85\%$), class (85 >

$x \geq 80\%$), and phylum ($80 > x \geq 75\%$). The read was assigned to an unclassified group when the similarity was below the cutoff point. Operational taxonomic units (OTUs) and rarefaction curves were generated with an identity cut off of 97%.

The diversity and species richness indices were calculated using the rRNA Database Project's pyrosequencing pipeline (http://pryo.cme.msu.edu/). The Shannon (Shannon and Weaver, 1949) and Simpson (Simpson, 1949) indices were calculated for each sample. For the variables of dominance hierarchy, relative abundance was calculated as the number of sequences divided by the total number of sequences per sample (%).

4.3 Results and discussion

4.3.1 Heavy metal concentrations in liquid fraction of CSTR

While heavy metals that exist in the solid fraction cannot directly affect the anaerobic process efficiency, heavy metals existing in the liquid fraction can inhibit bacterial activities when the concentration is higher than the inhibitory level (Lawrence and McCarty, 1965). The soluble heavy metal concentrations attributed from substrate (i.e., sunflower) during anaerobic digestion were investigated using a laboratory-scale CSTR. The substrate used in this study mainly contained five heavy metals such as Cd, Cu, Ni, Pb, and Zn. However, only soluble Cu and Zn were detected in solution, whereas Cd, Pb, and Ni were barely observed during the experimental periods. Cd, Pb, and Ni might have been removed from liquid fraction by adsorption onto biomass or precipitation with sulfur and hydroxide (Chen et al., 2008). The changes in soluble Cu and Zn concentrations within the reactor during operation period are shown in Fig. 4.2.

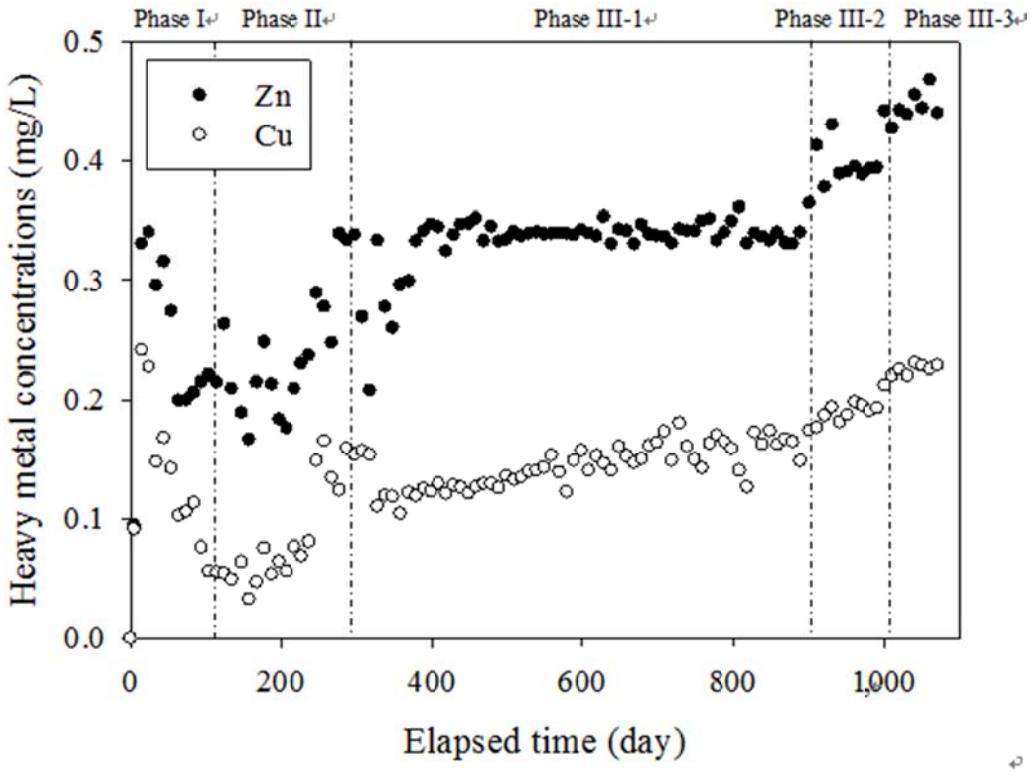


Figure 4.2 Soluble heavy metal concentrations in liquid fraction of CSTR

At the early phase of the reactor operation (i.e., Phase I and II), dissolved heavy metal concentrations in the reactor may have been unsettled due to the heavy metals originated from the inoculum. Their concentrations, however, were maintained under the same OLR during Phase III, and concentrations of Cu and Zn in liquid fraction increased in accordance to the increasing OLR. The average Cu and Zn concentrations in liquid fraction were highest within Phase III-3 and their concentrations were 0.225±0.004 and 0.445±0.012 mg/L, respectively. These concentrations of Cu and Zn in liquid fraction were below the reported inhibition levels. Previous studies have reported the inhibitory ranges of Cu and Zn concentrations in the anaerobic digestion process to be >1 mg/L for Cu^{2+} (Kouzeli-Katsiri and Kartsonas, 1986; Yenigün et al., 1996; Zayed and Winter, 2000) and >4 mg/L for Zn^{2+} (Kouzeli-Katsiri and Kartsonas, 1986; Yenigün et al., 1996; Zayed and Winter, 2000). When the heavy metal concentrations in solution exceed the inhibitory level, they can form unspecific compounds and affect anaerobic bacterial activities (i.e., cytotoxic effect) (Kavamura and Esposito, 2010), thus resulting in a failure of the process. Results of heavy metal concentrations with CSTR suggest that heavy metals in sunflower hardly affect anaerobic bacterial activities and growth under the conditions in this study.

4.3.2 Digestion performance

Although OLR applied in this study (i.e., 2.0 g VS/L/day at HRT of 20 days) was relatively high, considering that the OLR from other studies for anaerobic digestion of crop residues (Stewart et al., 1984; Wilkie et al., 1986), the anaerobic digester did not show significant difference in biogas production and methane content (Fig. 4.3). When the HRT was decreased from 30 days (Phase I) to 20 days (Phase III-1) (OLR increased from 1.0 to 1.5 g VS/L/day), biogas production in the reactor increased gradually, but the methane content maintained 49.25±5.79 - 51.31±2.02% by vol. of total biogas during the overall digestion process. When OLR was increased from 1.5 g VS/L/day (Phase III-1) to 2.0 g VS/L/day (Phase III-3) at same HRT as 20 days, the methane content was still stable (50.52±4.68 - 52.15±1.89% by vol. of biogas). Although OLR was increased from Phase III-1 to Phase III-3 under HRT of 20 days, biogas production was not significantly different. As illustrated in Fig. 4.3 (a), it can be witnessed that the daily biogas production remained relatively constant throughout Phase III. Yet, with the consideration of increased OLR during Phase III, it is clear that biogas production per gram of input substrate has in fact decreased. The average methane production rates decreased from 0.18 L/g VS at Phase III-1 to 0.16 and 0.14 L/g VS at Phase III-2 and 3, respectively. Incomplete substrate decomposition may have occurred when the OLR was raised beyond 1.5 g VS/L/day (at HRT of 20 days).

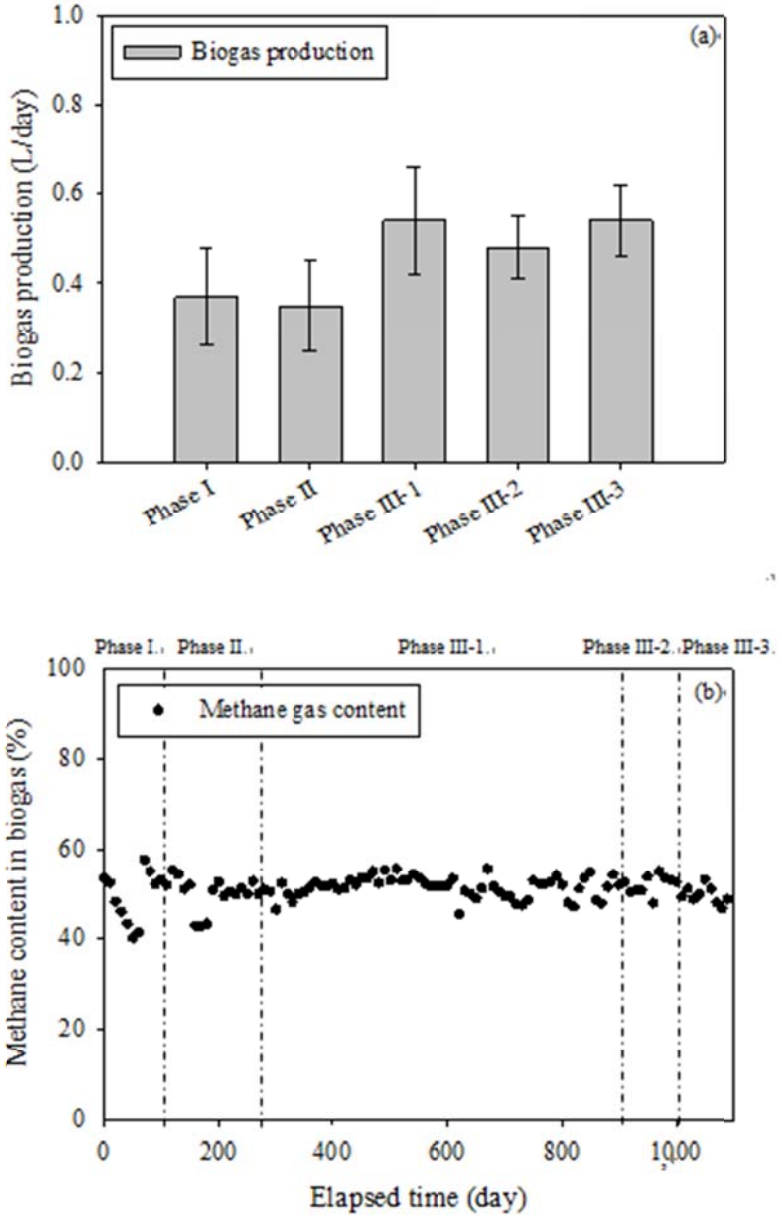


Figure 4.3 (a) Biogas production and (b) methane gas content in biogas over test periods

Total organic matter (TCOD) concentrations in the reactor tend to increase according to increased OLR (OLR increased from 1.5 to 2.0 g VS/L/day at same HRT), but the soluble organic matter (SCOD) concentrations in the reactor remained stable (Fig. 4.4). This is also probably due to incomplete decomposition of input substrate, subsequently leading to incomplete conversion of total organic matter and relatively unchanged soluble organic matter concentrations.

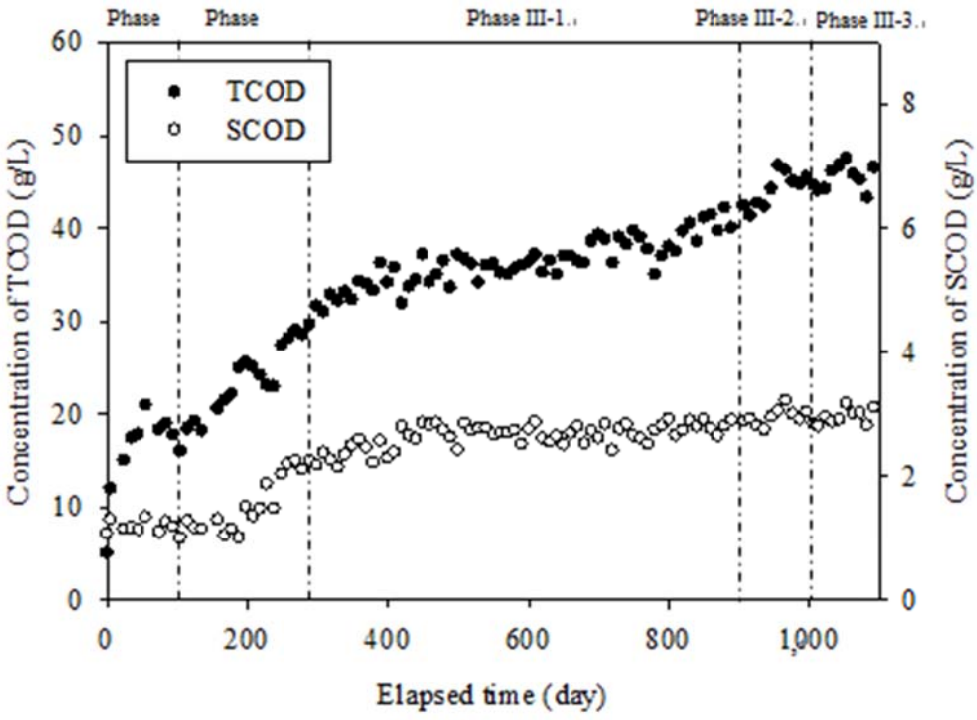


Figure 4.4 TCOD and SCOD values over test periods

VFAs concentration has been recognized as one of the most important parameters for reflect stability of the anaerobic digestion process (Chynoweth and Mah, 1971; Fischer et al., 1984; Hill and Bolte, 1989; McCarty and McKinney, 1961). Some indicators (i.e., alkalinity and pH) have correlated to the concentrations of VFAs in reactor (Hill et al., 1987). Accumulation of VFAs concentrations on the anaerobic digestion process, which could result in pH reduction and alkalinity consumption, is considered to be the main cause of reactor instability (Hill, 1982; Mosey and Fernandes, 1984). During the reactor operation period, total VFAs concentrations in reactor was maintained under the inhibition level (under 6,000 mg/L) reported by Siegert and Banks (2005) (Fig. 4.5).

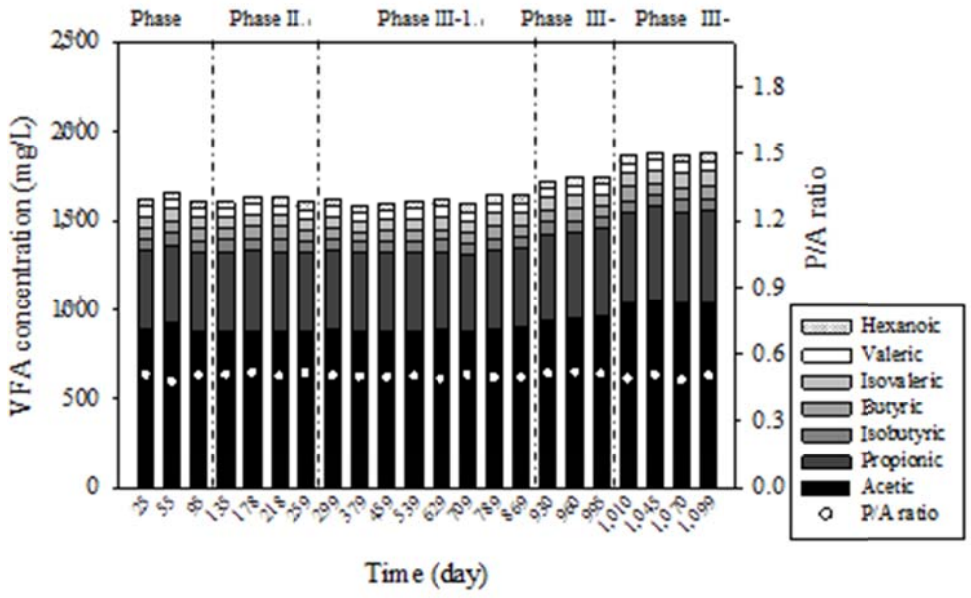


Figure 4.5 TVFAs concentrations over test periods

The concentration of propionic acid, which is most toxic to methanogen activities, was under 500 mg/L. The ratio of propionic acid to acetic acid (P/A ratio), which is one of the indicators for assessing stability of reactor performance, and remained about 0.5. Previous research reported that increase in propionic acid to acetic acid ratio (P/A) greater than 1.4 can suggest process inhibition and ultimate digester failure (Hill, 1982). The changes in alkalinity and pH value according to VFAs accumulation also suggest stable operation during all operation periods. Alkalinity and pH showed slight decrease, but maintained relatively stable at 2.6-3.2 g $CaCO_3$/L and 6.2-7.2 for alkalinity and pH, respectively (Fig. 4.6).

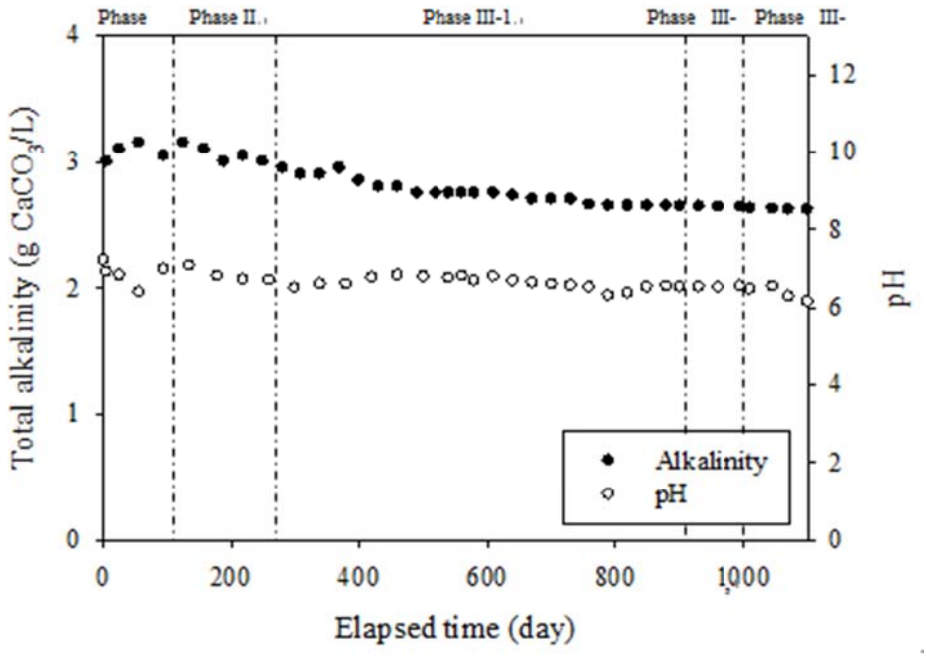


Figure 4.6 Total alkalinity and pH over test periods

These ranges are known to be favorable for substrate degradation and methane production during anaerobic digestion process (Anderson and Yang, 1992; Pohland and Bloodgood, 1963). Accumulation of VFAs, rapid increasing consumption of alkalinity, and drastic decrease in pH value were not observed during reactor operation. The results of reactor performance imply that anaerobic digester was not hindered by stress from extraneous input toxic substances and was stably operated.

4.3.3 Microbial community analysis

4.3.3.1 Pyrosequencing results and diversity indices

Microbial distribution from seed (i.e., raw sludge) and samples from phases I, II, III-1 and III-2 were identified by pyrosequencing to investigate the change of microbial communities during the anaerobic digestion process. The relative abundance and taxonomic distribution of the bacterial and archaeal communities in each sample were analyzed at the phylum, class, order, family, genus, and species levels along with unclassified sequences. Table 4.3 shows pyrosequencing data of bacterial and archaeal communities in the samples.

Table 4.3 Summary of pyrosequencing analysis of bacterial and archaeal communities in the samples

Samples	Total reads	OTUs [a]	Shannon index (H')	Simpson index (D')	Chao 1	Good's coverage (%)
Bacterial communities						
Seed	9,294	1,184	4.92	0.043	2,187	93.1
I	7,989	831	4.04	0.120	1,674	94.2
II	7,076	750	4.26	0.069	1,532	94.1
III-1	9,079	872	4.14	0.075	1,551	94.8
III-2	5,514	463	3.72	0.083	969	95.3
Archaeal communities						
Seed	18,519	167	1.82	0.395	205	99.8
I	15,880	128	2.93	0.077	153	99.8
II	13,024	87	2.97	0.078	98	99.9
III-1	17,552	62	2.06	0.237	80	99.9
III-2	15,396	58	2.13	0.178	64	99.9

[a] Operational taxonomic units.

The 16S RNA gene survey produced a total of 38,952 sequences for bacterial communities. Total reads were obtained as 9,294, 7,989, 7,076, 9,079, and 5,514, and operational taxonomic units (OTUs) using a 3% sequence dissimilarly cut-off value showed 1,184, 831, 750, 872, and 463 in seed, phase I, II, III-1, and III-2 sample, respectively. Observed OTUs were significantly higher than estimated Chao 1, indicating that additional bacterial phylotypes could be observed as 1,003 (seed), 843 (I), 782 (II), 679 (III-1), and 506 (III-2). Individual rarefaction curves of each of the samples also demonstrated a similar result, and the curve approached an asymptote, but did not reach a saturation phase (Fig. 4.7 (a)). However, the Good's coverage, which indicates sampling completeness, suggests that most of bacterial phylotypes present in each samples were detected in this study. For archaeal communities, 9,294, 7,989, 7,076, 9,079, and 5,514 of total reads were obtained in seed, phase I, II, III-1, and III-2 sample, respectively with a total of 80,371 sequence. OTUs were observed as 167, 128, 87, 62, and 58 in each sample, and observed OTUs were similar to the estimated Chao 1. The Good's coverage of archaeal communities showed over 99%, and the individual rarefaction curves reached to saturation phase, indicating that this result could fully cover the archaeal communities of samples (Fig. 4.7 (b)).

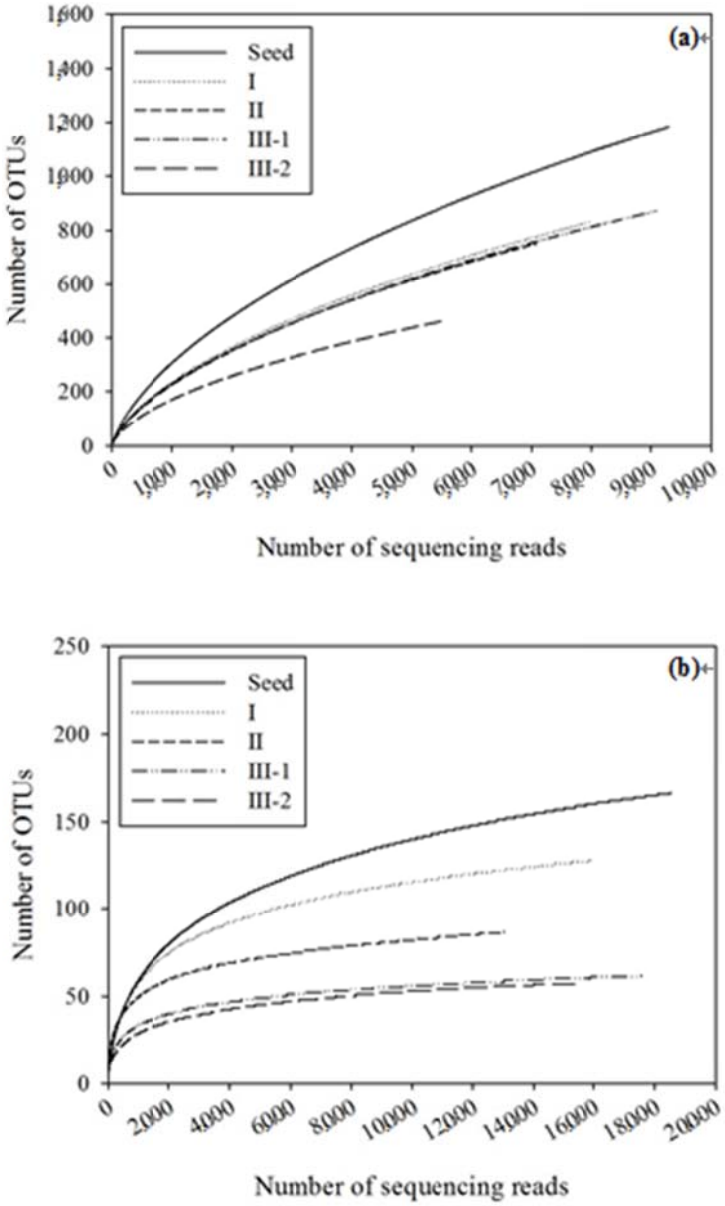


Figure 4.7 Rarefaction curves of 16s rRNA gene sequencing reads of microbial diversity in each sample during the anaerobic digestion. Fig (a) and (b) indicated bacterial and archaeal communities, respectively.

The diversity indices were estimated by Shannon index (H') and Simpson index (D') based on OTUs data set. The microbial diversity is positively correlated with Shannon index (H'), representing the species abundance, and has negative correlation with the Simpson index (D') (Morris et al., 2014; Song et al., 2014). The Shannon index (H') of bacterial communities were higher than those of archaeal communities. The Shannon index (H') ranged from 3.72 to 4.92 with the highest H' in seed for bacterial communities, and ranged from 1.82 to 2.97 with the highest H' in phase II for archaeal communities. The bacterial and archaeal diversity showed a slightly decreasing trend over time. The Simpson index (D') showed some variation in the range from 0.043 to 0.120 with the highest D' in phase I, and also indicated a decreasing trend over time for bacterial comminutes. The Simpson index (D') of archaeal communities ranged from 0.077 to 0.395. The highest value of 0.395 was observed in seed, sharply dropped to 0.077 and 0.078 in phase I and II, and then increased to 0.237 and 0.178 in phase III.

4.3.3.2 Taxonomic distribution of the microbial communities

For bacterial communities, the phylogenetic classification of sequences from samples at the phylum and class level is summarized in Fig. 4.8.

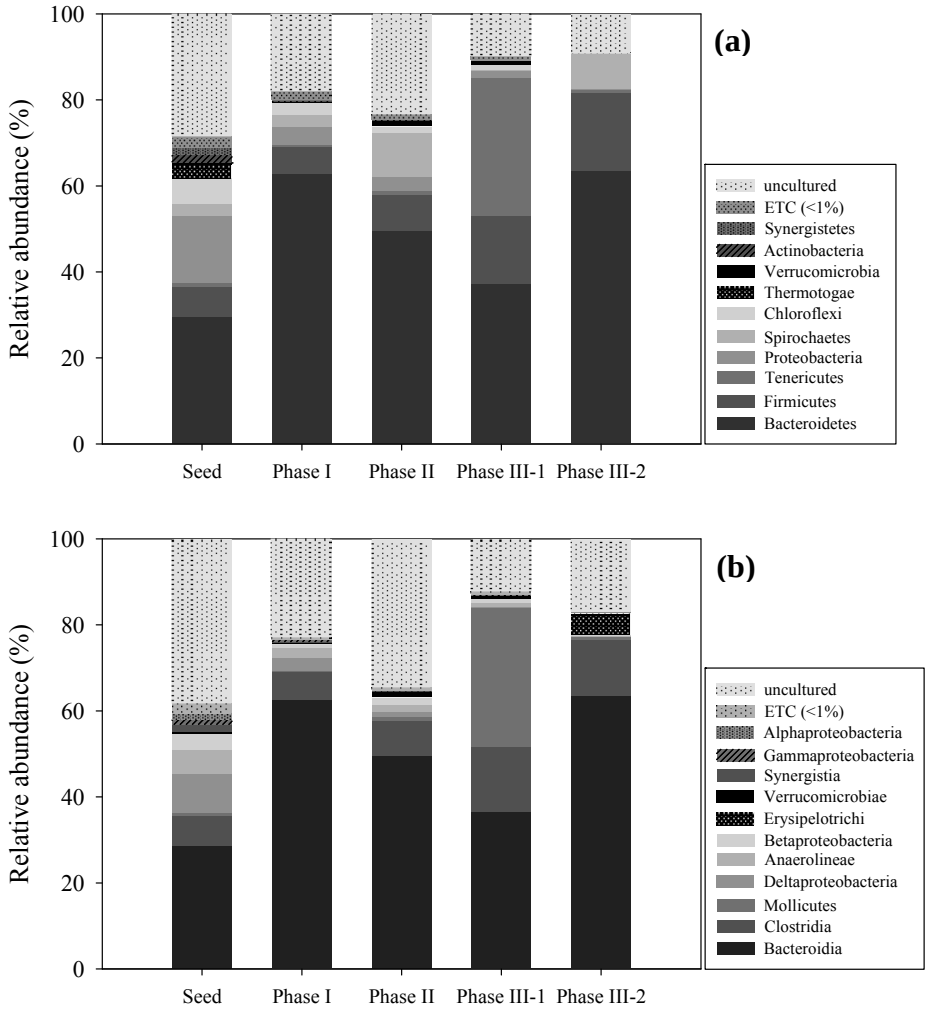
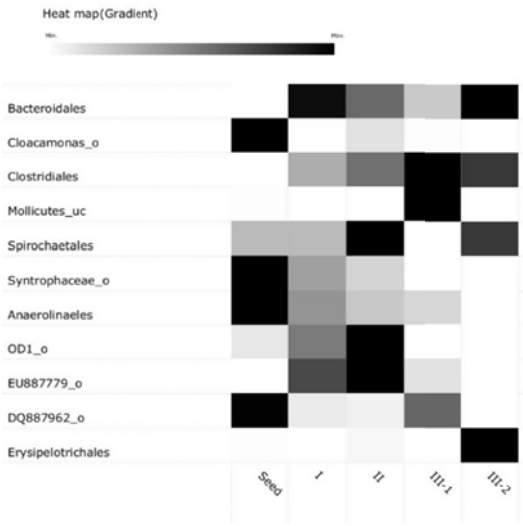


Figure 4.8 The changes in bacterial communities at the (a) phylum and (b) class level

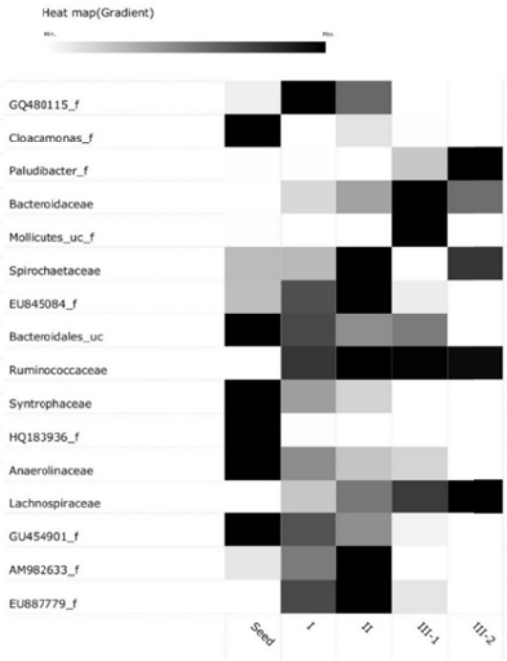
The major phyla groups in samples during anaerobic digestion were *Bacteroidetes* (29.6-63.6%), *Firmicutes* (6.5-18.2%), *Proteobacteria* (0.4-15.4%), *Tenericutes* (0.3-32.3%), *Chloroflexi* (0.3-6.0%), and *Spirochaetes* (0.25-10.1%). The remaining phylotypes were associated with *Thermotogae* (0-3.2%), *Verrucomicrobia* (0.3-1.3%), *Actinobacteria* (0.1-1.8%), *Synergistetes* (0-1.8%), and unknown bacterial group (8.8-28.4%) with an exclusion of bacteria with less than 1% of relative abundance. The sequences classified at the class level mainly composed of *Bacteroidia* (28.6-63.5%), *Clostridia* (6.4-15.3%), *Deltaproteobacteria* (0.1-8.9%), *Anaerolinea*e (0.2-5.5%), and *Betaproteobacteria* (0.2-3.8%), but the unclassified group was relatively abundant (12.1-38.1%). The most abundant phylum in all samples was *Bacteroidete*s, which is frequently detected in anaerobic reactors with important roles as fermenters and acidogens (Jang et al., 2014). Phylum *Firmicute*s also steadily increased over time, ranging from 7.1 to 18.2%. *Firmicutes* are known to be involved in hydrolyzing polymers (e.g., cellulose, lignin), and producing organic acids as metabolic endpoints. Classes *Bacteroidales* and *Clostridia*, which belong to phylums *Bacteroidetes* and *Firmicutes*, were relatively abundant during the overall digestion. Typically, most bacteria that belong to *Bacteroidetes* can produce various lytic enzymes and acetic acid during the degradation of organic materials (Riviere et al., 2009; Robert et al., 2007). Numerous bacteria belonging to *Bacteroidales* can efficiently degrade complex organic matters and ferment lactic or acetic acid to H_2 and CO_2 (Jang et al., 2014; Wirth et al., 2012). The most dominant bacteria at the order level were *Bacteroidales* (28.4-63.5%),

which originate from the class *Bacteroidales* and the phylum *Bacteroidetes*. *Clostridiales* (3.1-11.9%) are known to create cellulosomes, which are intensively involved in the anaerobic digestion of recalcitrant cellulose and support acetogens and methanogens with compounds necessary for their growth (Ziganshin et al., 2013). *Syntrophaceae, Anaerolinaceae, Anaerolinaceae*, and *Bacteroidaceae* were mainly discovered at the family level. At the genus level, most of the bacterial sequences could not be assigned, remaining unknown species (66.4-93.9%). 24.4% of *Cloacamonas*, known as H_2-producing bacteria, was found in seed, but steadily decreased over time. A dramatic increase of genus *Bacteroides* was observed, ranging from 0.3 to 10%, during anaerobic digestion. These bacteria have enormous potential for degradation and utilization of complex carbohydrate (De Vos et al., 2004). An approximate number of 984 species were assigned based on ExTaxone database, and most sequences (>95%) remained as unknown bacteria. More than 1% of the found species were *Cloacamonas acidaminovorans* and *Bacteroides graminisolvens*. Moreover, *Clostridium* sp. (e.g., *Clostridium xylanolyticum, Clostridium puniceum, Clostridium aldrichii,* and *Clostridium celatum*) which can degrade the plant biomass were also found (Ziganshin et al., 2013). Heat map analyses at the order, family, genus, and species levels are shown in Fig. 4.9.

(a)


Heat map(Gradient)
Bacteroidales
Cloacamonas_o
Clostridiales
Mollicutes_uc
Spirochaetales
Syntrophaceae_o
Anaerolinaeles
OD1_o
EU887779_o
DQ887962_o
Erysipelotrichales
Seed
I
II
III-1
III-2

(b)


Heat map(Gradient)
GQ480115_f
Cloacamonas_f
Paludibacter_f
Bacteroidaceae
Mollicutes_uc_f
Spirochaetaceae
EU845084_f
Bacteroidales_uc
Ruminococcaceae
Syntrophaceae
HQ183936_f
Anaerolinaceae
Lachnospiraceae
GU454901_f
AM982633_f
EU887779_f
Seed
I
II
III-1
III-2

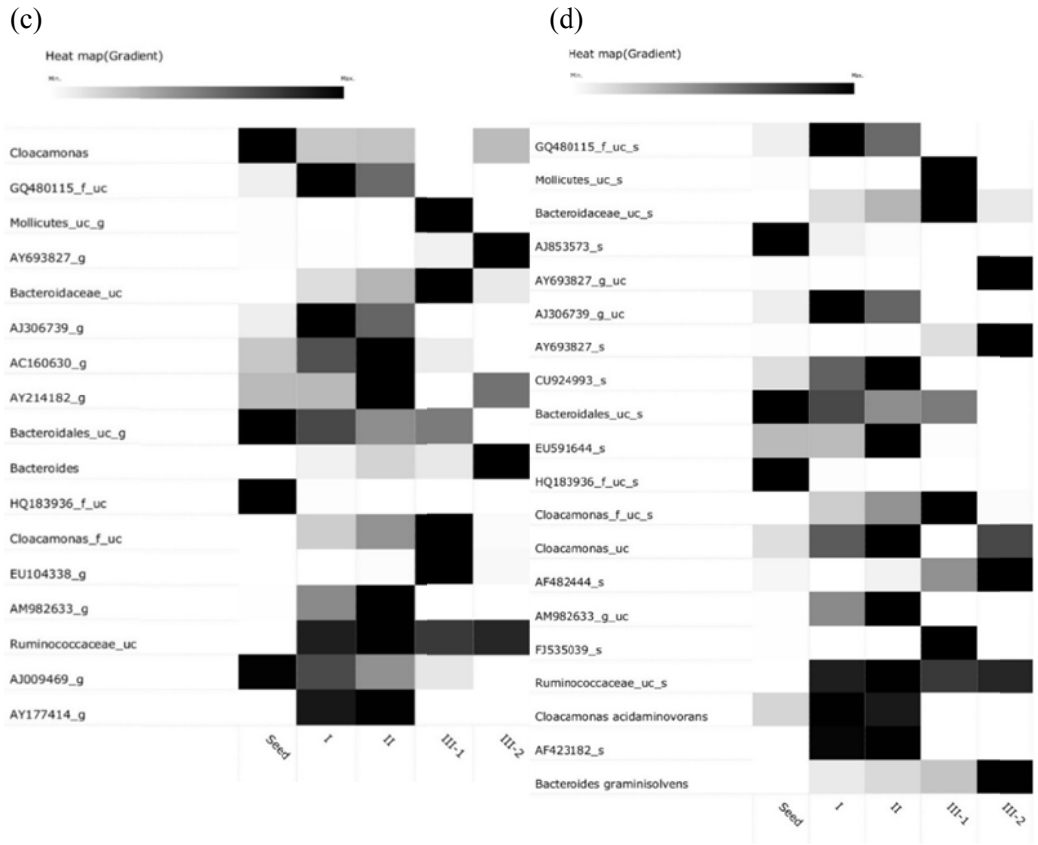


Figure 4.9 Heat map analysis for bacterial communities at the (a) order, (b) family, (c) genus, and (d) species level

The archaeal communities had a relatively unsophisticated composition compared to bacterial communities. Two phylum *Euryarchaeota* (54.2-98.8%) and *Crenarchaeota* (1.2-45.8%) were detected in all samples. *Methanobacteria* (10.2-69.7%), *Thermoplasmata* (8.5-29.4%), and *Methanomicrobia* (9.8-18.2%) were obtained including unknown archaeal groups (1.2-67.1%) at the class level. The dominant archaeal sequences were *Methanobacteriales* (10.2-69.7%), *Methanosarcinales* (9.3-17.1%), and *Methanomicrobiales* (0.2-1.1%) at the order level. Two orders *Methanobacteriales* and *Methanosarcinales* had a similar proportion (10.2 and 13.2%, respectively). However, only *Methanobacteriales* had significantly increased during anaerobic digestion, and more than 60% was found at phase III (i.e., phase III-1 and III-2). These changes demonstrate that methanogenic communities shift from aceticlastic (*e.g., Methanosarcinales*) to hydrogenotrophic methanogens (e.g., *Methanobacteriales and Methanomicrobiales*) with high increase in the proportion of syntrophic bacterial communities (Jang et al., 2014). The archaeal sequences at the family level mainly composed of *Methanobacteriaceae* (10.2-69.7%), *Methanosarcinaceae* (0.1-16.8%), *Methanosaetaceae* (0.2-15.3%), and unknown archaeal groups (20.4-75.9%). At the genus level, Methanobacterium (6.6-69.7%) was the major sequence, and their portion sharply increased from 6.6% with seed to 69.7 and 57.4% within phase III. More interestingly, genus *Methanosarcina* also increased from 0.1% within seed to 16.8% within phase III-2. *Methanosarcina* populations are known to effectively buffer against fluctuations in substrate availability, preventing

accumulation or shock loading of acetic acid (Conklin et al., 2006; FitzGerald et al., 2015). Although no significant changes were directly observed in terms of VFAs concentrations, alkalinity, and pH from digestion performance, potential problems on organic acid accumulation and organic loading shock may be conjectured. The increase in genus *Methanosarcina* may indicate possible process instability and organic acids accumulation. *Methanobacterium formicicum* (0-54.8%), *Methanomassiliicoccus intestinalis* (7.2-22.2%), *Methanosarcina barkeri* (0-15.8%), *Methanosaeta concilii* (0.2-14.9%), *Methanobacterium beijingense* (0.4-10.0%), *Methanobacterium petrolearium* (0-16.2), *Methanosarcina vacuolata* (0-9.8%), *Methanobacterium subterraneum* (0-5.2%), and *Methanobacterium palustre* (0-4.1%), were observed in archaeal communities at the species level. Table 4.4 shows the relative abundance in archaeal communities over time at the (a) phylum, (b) class, (c) order, (d) family, (e) genus, and (f) species levels.

Table 4.4 The relatively abundance of the predominant phylogenetic groups in in archaeal communities. Relative abundance is defined as the number of sequences affiliated with that taxon divided by the total number of sequences per sample (%). Genera making up less than 1% of the total composition in both libraries are defined as "others".

Sequences	Relative abundance (%)				
	Seed	Phase I	Phase II	Phase III-1	Phase III-2
(a) Phylum					
Euryarchaeota	98.4	76.6	54.2	95.0	98.8
Crenarchaeota	1.5	23.2	45.8	5.0	1.2
unknown	0.2	0.1	0.0	0.0	0.0
(b) Class					
Methanobacteria	10.2	22.9	10.9	69.7	57.4
Thermoplasmata	8.5	22.2	29.4	15.5	23.1
Methanomicrobia	14.3	15.8	13.8	9.8	18.2
unknown	67.1	39.2	45.8	5.1	1.2
(c) Order					
Methanobacteriales	10.2	22.9	10.9	69.7	57.4
Methanosarcinales	13.2	15.4	13.6	9.3	17.1

Methanomicrobiales	1.1	0.4	0.2	0.5	1.1
unknown	75.5	61.4	75.3	20.6	24.3
(d) Family					
Methanobacteriaceae	10.2	22.9	10.9	69.7	57.4
Methanosarcinaceae	0.1	0.1	12.6	7.2	16.8
Methanosaetaceae	13.1	15.3	0.9	2.1	0.2
others	0.7	0.1	0.2	0.6	1.2
unknown	75.9	61.7	75.3	20.4	24.3
(e) Genus					
Methanomassiliicoccus	8.0	22.2	8.9	9.3	7.2
Methanosarcina	0.1	0.0	12.6	7.2	16.8
Methanosaeta	13.1	15.3	0.9	2.1	0.2
Methanobrevibacter	1.9	0.1	0.1	0.0	0.0
others	1.4	0.5	0.2	0.5	1.1
unknown	69.1	39.6	66.4	11.3	17.2
(f) Species					
Methanobacterium formicicum	0.1	0.0	4.7	54.8	46.6

Methanomassiliicoccus intestinalis	7.9	22.2	8.9	9.3	7.1
Methanosarcina barkeri	0.0	0.0	2.7	6.7	15.8
Methanosaeta concilii	5.8	14.9	0.9	2.1	0.2
Methanobacterium beijingense	0.4	0.4	1.1	10.0	9.0
Methanobacterium petrolearium	2.9	16.2	1.4	0.1	0.0
Methanosarcina vacuolata	0.0	0.0	9.8	0.4	1.0
Methanobacterium subterraneum	2.2	5.2	0.6	0.5	0.0
Methanobacterium palustre	0.3	0.0	1.6	4.1	1.8
others	3.2	0.5	1.6	0.1	0.0
unknown	77.1	40.6	66.7	11.9	18.4

4.4 Summary

Anaerobic digestion of heavy metal-containing substrate must be applied carefully due to the effect of endogenous heavy metals in substrate on anaerobic digestion. In this study, the feasibility of anaerobic digestion for heavy metal-containing crop residues harvested from contaminated sites was investigated and the bacterial and methanogenic archaeal communities were observed during the digestion. Adverse effects of heavy metals on reactor performance was not observed (i.e., biogas production, methane content in biogas, organic matter decomposition, VFAs concentration, alkalinity, and pH). From the results of microbial community analysis during anaerobic digestion through pyrosequencing, most of the observed microbial sequences were commonly found in anaerobic reactors for cellulosic biomass, implying that the communities were conformed to the substrate. Stable reactor operation represented that the balance of microbial metabolism was maintained appropriately. Thus, the microorganisms in reactor did not suffer from extraneous toxic substances (i.e., existence of heavy metal in reactor). Interestingly, the proportion of microbial population related to organic acid accumulation increased. This demonstrated that the reactor might be affected by operating conditions such as OLR and HRT. Therefore, when accompanied proper design of the practical operation parameters (i.e., OLR and HRT), anaerobic digestion of heavy metal-containing crop residues from phytoremediation sites can be an appropriate approach without adverse effects of heavy metals.

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CHAPTER 5
RELEASING CHARACTERISTICS OF HEAVY METALS FROM CROP RESIDUES UNDER ANAEROBIC CONDITION

5.1 Introduction

Anaerobic digestion for crop residues has been applied as a plausible technology in terms of renewable energy production, byproduct utilization, and agricultural wastes reduction (Zhang et al., 2013). When crop residues are harvested in contaminated sites, abandoned lands may be efficiently utilized along with the benefit of remediating contaminated soils (Evangelou et al., 2012). However, anaerobic digestion of crop residues from heavy metal contaminated sites must be approached carefully due to endogenous heavy metals in crop residues. Existence of heavy metals can have an adverse impact on anaerobic digestion processes. There have been many researches that have emphasized inhibitory effects of soluble metal concentration (i.e., ionic form in the solution), type of metal species, and amount/distribution of biomass in the digester. The most important is the amount of heavy metals that exist as ionic form in the solution, which is known to directly affect anaerobic bacterial activity. Therefore, research on the releasing characteristics and fate of heavy metals from crop residues during anaerobic degradation is required for better understanding of anaerobic

digestion of heavy metals-containing crop residues.

Heavy metals exert significant roles in biochemical reactions because of their cytotoxic effects. The toxic effect of heavy metals is attributed to the chemical binding of heavy metals to the enzymes of bacteria (Brady and Duncan, 1994; Krishna and Gilbert, 2014) and subsequent disruption of enzyme structures and activities (Li and Fang, 2007). Although the heavy metal concentrations contained in biomass is low, they are not biodegradable and can accumulate to potentially toxic concentrations in anaerobic digester (Krishna and Gilbert, 2014). However, most of the researchers using heavy metal-containing biomass mainly investigated in terms of biogas generation. There is few study that examines the detailed releasing characteristics of heavy metals in biomass. The releasing characteristics of heavy metals from biomass should be considered because soluble heavy metals, and not the total heavy metal amounts, can inhibit the anaerobic digestion process. Heavy metals can be mainly released from biomass through the degradation of biomass by bacteria in anaerobic digester, and subsequently become soluble heavy metals.

Moreover heavy metals have been reported to be released into solution as soluble forms and the reaction (i.e., adsorption and precipitation) of released heavy metals can occur in anaerobic digestion (Lange and Weber, 1993; Shen et al., 1993). Several studies reported that heavy metal inhibition depends upon chemical forms of heavy metals in anaerobic digester. Oleszkiewicz and Sharma (Oleszkiewicz and Sharma, 1990) demonstrated that only soluble form can be considered to predict the inhibitory

response of heavy metals in anaerobic digester. Bhattacharya et al. (Bhattacharya et al., 1995) also concluded that heavy metal toxicity can be strongly dependent upon the free ionic concentration of the metal in solution rather than the total metal concentration. Concentration of soluble heavy metals needs to be monitored carefully due to its toxicity to anaerobic bacteria (Li et al., 2015).

The aim of study was to investigate the releasing characteristics and fate of heavy metals that are released from heavy metal-containing crop residues during anaerobic degradation. The ultimate amounts of heavy metals in solution, which depend on the fate of each heavy metal species, were demonstrated. In this study, heavy metal releasing trend from biomass according to biomass degradation and reaction of released heavy metals in solution were investigated using lab-scale batch tests and Visual MINTEQ equilibrium prediction model.

5.2 Materials and methods

5.2.1 Characterization of heavy metal-containing biomass

In this study, sunflower (i.e., *Helianthus annuus*) was used as the biomass. Sunflower is the most frequently used plant for remediation of heavy metal contaminated site for its high heavy metal accumulating capacity (Lee et al., 2013; Lone et al., 2008), and its relatively high biomass production compared to other plants (Zhuang et al., 2005). In addition, sunflower could be easily cultivated in various soil textures of Korea. The sunflower used in this study was grown for 120 days in heavy metal contaminated site near an abandoned mine at Jecheon-si, Chungcheongbuk-do, Korea. This site was contaminated with 3.98 mg-Cd, 155.13 mg-Pb, 28.47 mg-Ni, 29.17 mg-Cu, and 236.25 mg-Zn/kg-soil. Physicochemical properties of soils in site showed organic matter content of 2.51% by dry wt., pH of 6.9, and texture of silt loam.

The harvested biomass were carefully removed from the soils, and washed with distilled water to remove soil particles. For heavy metal analysis, the crop residues were oven-dried completely to remove moisture until the constant weight of crop residues was maintained. Oven-dried crop residues were ground into fine particles and then digested with a solution of HNO_3, H_2O_2, and distilled H_2O (9:1:1, v/v/v), using a microwave digester (MSP1000, CEM, USA) according to the U.S. Environmental Protection Agency (U.S. EPA) 3052 method for heavy metal analysis. After digestion,

the volume of each sample was adjusted to 25 mL with distilled water. The concentrations of heavy metal in crop residues were determined by ICP-OES (iCAP 7400, Thermo, USA). Proximate analysis (ASTM standard test method E871-82, E872-82, and E1755-01), elemental composition analysis (e.g., carbon, hydrogen, oxygen, sulfur and nitrogen contents), and structural analysis (AOAC official method 973.18 and 2002.04) were also conducted. The characterization of sunflower used in this study is shown in Table 5.1.

Table 5.1 The physicochemical characterization of biomass (i.e., sunflower) used in this study

Characterization		Value	Unit
Proximate analysis	Moisture content	60.0	% by wt. (wet basis)
	Volatile/Total solid	84.0	% by wt. (dry basis)
Elemental analysis	C	41.9	% by wt. (dry basis)
	H	5.40	
	N	2.06	
	S	0.22	
	O (diff.)	42.9	
	Ash	7.47	
Heavy metal concentration	Cd	3.21±0.003	mg/kg crop residue (dry basis)
	Pb	13.1 ±0.005	
	Zn	56.0 ±0.018	
	Ni	1.45±0.004	
	Cu	26.3 ±0.006	
Structural analysis	Cellulose	65.0	% by wt. (dry basis)
	Hemicellulose	24.3	
	Lignin	10.7	

5.2.2 Biomass degradation and heavy metal releasing during anaerobic digestion

Lab-scale anaerobic batch tests were conducted to investigate the biodegradation of biomass and releasing characteristics of heavy metals from biomass (Fig. 5.1). In order to investigate the biodegradation of biomass and the amount of heavy metals released according to biomass degradation, a series of parallel 250 mL-serum bottles (working volume = 100 mL) were seeded with anaerobic sludge obtained from the waste-water treatment plant. Three grams of heavy metal-containing sunflower were added to each bottle as biomass. The bottles were flushed continuously with N_2 gas for one minute to make anaerobic conditions, after which they were sealed with butyl rubber stopper and capped with aluminum crimp. A constant internal temperature of 35±1°C and 150 rpm were achieved in a temperature controlled mechanical shaker. Each bottle was irregularly sampled in time-series, and pH values, volatile solid (VS) contents, distribution of carbon contents, heavy metals in liquid phase were analyzed.

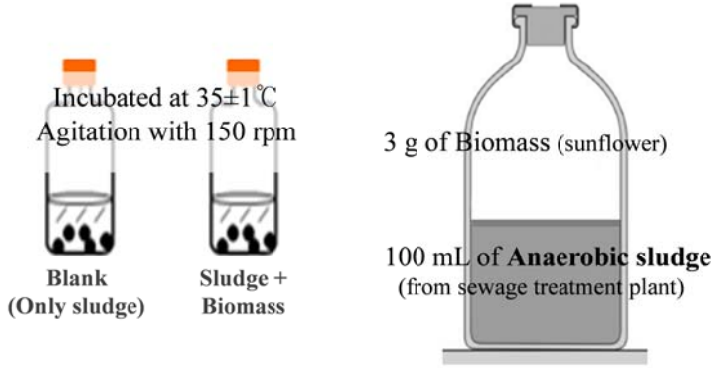

Figure 5.1 Experimental set up for biomass degradation and releasing characteristics of heavy metal

The pH of the solution was measured using a pH meter (Orion Star A216, Thermo, USA), fitted with a combined glass-reference electrode. Degradation of biomass was estimated via changes in VS removal ratio and carbon balance. VS contents were analyzed according to the American Society for Testing and Materials (ASTM) standard test method E872-82. VS removal was calculated with Eq. 5.1.

$$VS_{removed} = 1 - \frac{VS_t \cdot (1 - VS_i)}{VS_i \cdot (1 - VS_t)} \quad \text{Eq. 5.1}$$

$VS_{removed}$ is the VS degree of degradation (% by wt.), VS_i is the initial VS concentration of input biomass (% by wt. of TS), and VS_t is the VS concentration of the each bottle at point of sampled time t (% by wt. of TS).

Changes in carbon balance were determined by measuring the amount of consumed carbon of biomass (i.e., solid phase) that was converted into CH_4-carbon and CO_2-carbon (i.e., gas phase) via carbon in solution (i.e., liquid phase). The carbon contents in solid (biomass) and liquid phase were measured by TOC analyzer (TOC-VCPH, Shimadzu, Japan), and those in gas phase (CH_4 and CO_2) were analyzed by Gas Chromatography (ACME 6100, Younglin, Korea) with a thermal conductivity detector (TCD) operated at 120°C, and injector and oven temperatures of 120 and 35°C, respectively. Helium was used as the carrier gas. The initial carbon distributions were assumed to have mainly remained in solid phase (i.e., biomass). The remaining carbon amount in biomass was considered as solid phase, elevated carbon amount in solution

as liquid phase, and carbon amount in generated gas (i.e., CH_4 and CO_2) as gas phase. Therefore, under the assumption that decreased carbon amount in solid phase is relocated to liquid and gas phases, carbon mass balance can be maintained. Heavy metals in solid and liquid phase in each bottle were analyzed by ICP-OES (iCAP 7400, Thermo, USA). Heavy metal concentrations in solid phase were determined according to the US EPA 3052 method (US EPA, 1996). The solid phase was obtained after centrifugation and filtration of the mixtures within each bottle.

5.2.3 Prediction of heavy metal existing form after releasing by Visual MINTEQ 3.0

The amount of heavy metals precipitated were predicted with Visual MINTEQ 3.0 (U.S. EPA) to examine the fate of released heavy metals under anaerobic condition. MINTEQ is an equilibrium speciation model that can be used to calculate the equilibrium composition of dilute aqueous solutions in the laboratory or in natural aqueous systems (Fig. 5.2).

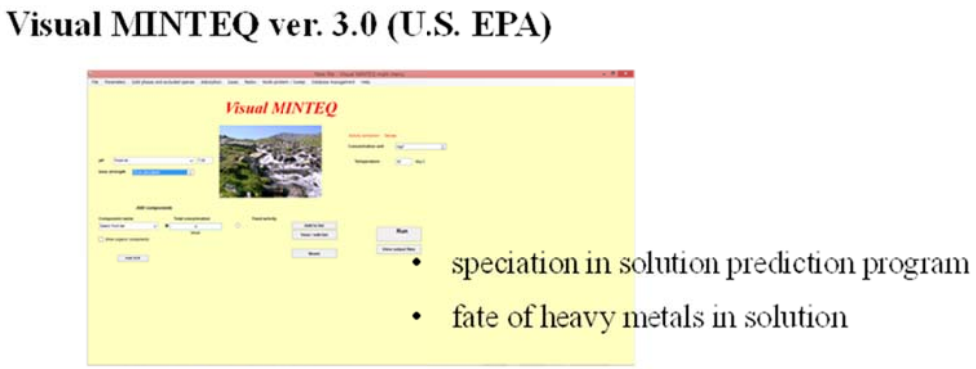

Figure 5.2 Introduction of Visual MINTEQ 3.0 (U.S. EPA)

The model is useful for calculating the equilibrium mass distribution among dissolved species, adsorbed species, and multiple solid phases under a variety of conditions including a gas phase with constant partial pressures. The releasing was usually solubility controlled and dependent on precipitation / dissolution / complexation equilibrium, which may be estimated based on these equilibrium reactions. Input mass concentration for each component (Cd^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+}, and

Zn^{2+}) is based on the initial concentration in crop residue. Visual MINTEQ was run for predicting pH dependent releasing behavior and stabilization process with addition of chemical agent in the absence of surface complexation reactions. According to the information referenced, the species was supplied, and the thermo dynamical parameter of some compounds was modified in the database.

5.2.4 Biosorption test under anaerobic condition

Batch tests were performed to measure the amount of heavy metals adsorbed onto sorbents (i.e., sludge and crop residues). The sorption ability of sorbents (i.e., sludge, crop residue, and mixture of sludge and crop residues) were performed using a mixed heavy metals solution containing Cd^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+}, and Zn^{2+} (Fig. 5.3).

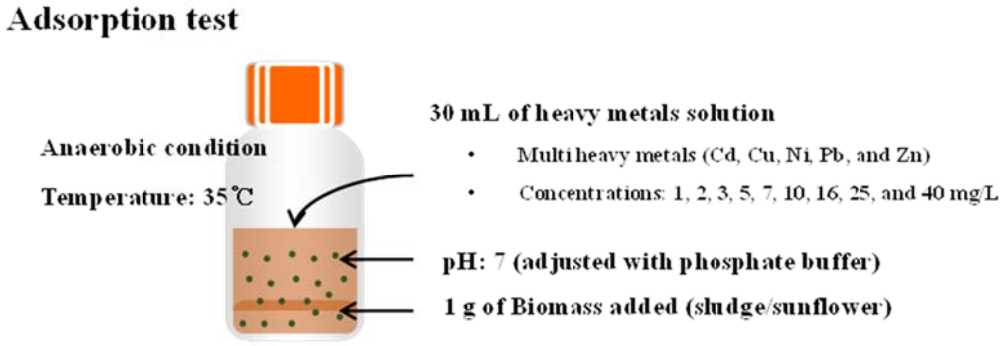

Figure 5.3 Experimental set up for adsorption tests

The concentration of each metal in the solution was adjusted as 1, 2, 3, 5, 7, 10, 16, 25, and 40 mg/L and pH of solution was also adjusted at a value of seven using phosphate buffer. About one gram of each sorbent was added into screw top Teflon tube and mixed with 30 mL of the heavy metal solution at 35±1°C. The tubes were flushed continuously with N_2 gas for one minute to make anaerobic conditions. After shaking in a mechanical shaker until equilibrium, the test tubes were withdrawn and filtered immediately through 0.45 μm pore size glass fiber filter. The heavy metal concentrations in the filtrates were determined using ICP-OES (iCAP 7400, Thermo, USA). The adsorbed heavy metal concentrations were calculated based on the difference between the initial and final metal concentrations in the supernatant. Test tubes without the sorbents (sludge, crop residue, and mixture of sludge and crop residues) or the sorbates (heavy metals) were included as experimental controls.

5.3 Results and discussion

5.3.1 Biodegradation of biomass under anaerobic condition

From lab-scale anaerobic batch test, the biodegradation of biomass (i.e., sunflower) under anaerobic digestion was determined by two methods: VS removal of biomass and carbon balance analysis. Fig. 5.4 shows the changes of biodegradation ratio (% by wt.) of biomass determined by changes in VS removal ratio for 77 days.

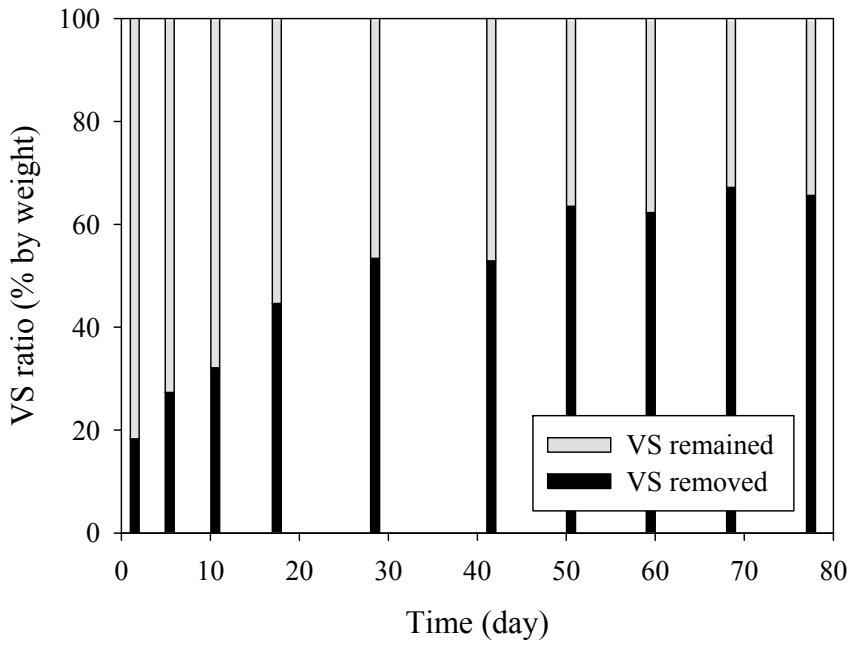

Figure 5.4 The changes of biodegradation ratio (% by wt.) of biomass over times determined by VS removal ratio

The biodegradation ratio seems to be similar after 50 days and complete degradation was observed at 77th day. In case of VS removal of biomass, the degradation of biomass gradually increased to approximately 60% by wt. over 77 days. The results of carbon balance change are has also been represented in Fig. 5.5. For 77 days, carbon of biomass gradually decreased from 98 to about 50% (by wt.). Carbon in liquid phase and gas phase increased from 2 to 10% and 0 to 40% (by wt.), respectively. Approximately 55% by wt. of carbon in biomass (i.e., solid phase) was degraded, and was recovered to gas phase (i.e., CH_4 and CO_2).

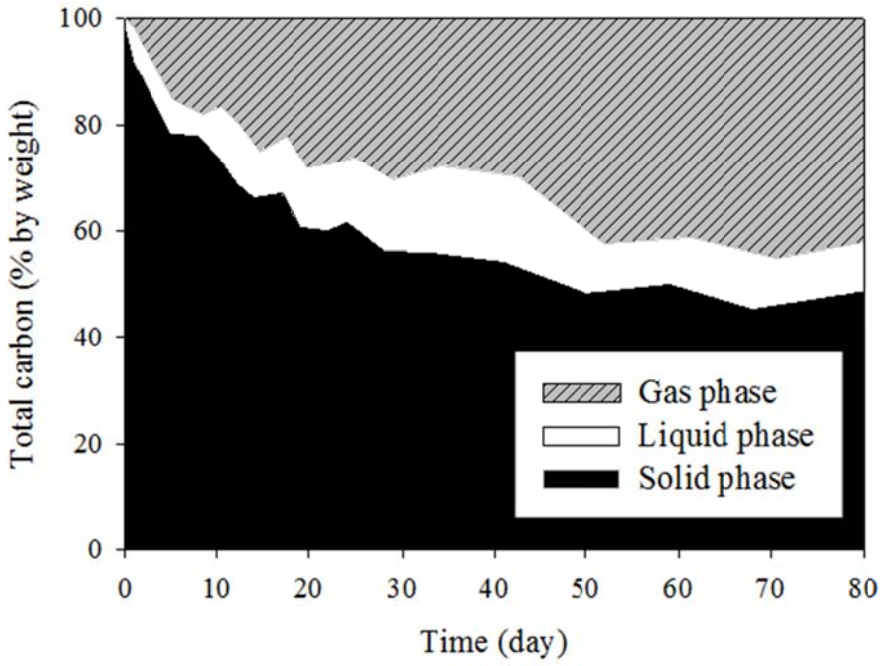

Figure 5.5 The changes of biodegradation ratio (% by wt.) of biomass over times determined by carbon balance

As a result, approximately 60% by wt. of biomass (i.e., sunflower used as substrate in this study) degradation was observed according to VS removal under anaerobic conditions for 77 days. This degradation efficiency (% by wt.) was comparable to the values of other ligno-cellulosic biomass. Lehtomäki et al. (Lehtomäki and Björnsson, 2006) reported that about 46% of willow, 59% of grass, and 96% of sugar beet were degraded according to VS reduction for 85, 50, and 55 days, respectively, under mesophilic anaerobic condition. Akinshina et al. (Akinshina et al., 2012) also revealed that approximately 50-70% of *Climacoptera lanata* and *Panicum coloratum* were decomposed under anaerobic condition at 35°C for 30 days. Sufficient decomposition of the biomass used in this study (i.e., sunflower) during the experimental period was determined, and thus, it was considered as a moderately biodegradable organic substrate.

5.3.2 Heavy metals releasing from biomass according to biodegradation

The amounts of soluble heavy metals, which were released from biomass (i.e., sunflower) under anaerobic conditions, were obtained from lab-scale anaerobic batch test of biomass degradation. The biomass used in this study contained five heavy metals: Cd, Cu, Ni, Pb, and Zn. However, only soluble Cu and Zn were detected in solution, whereas Cd, Ni and Pb were barely observed during experimental periods. Fig. 5.6 shows the ratio between observed mass and input mass of Cu and Zn and the VS removal ratio for 77 days. 0.079 mg of Cu and 0.168 mg of Zn were initially contained in input total biomass. About 40% by wt. of the initial amount of Cu and Zn contained in biomass was observed in solution.

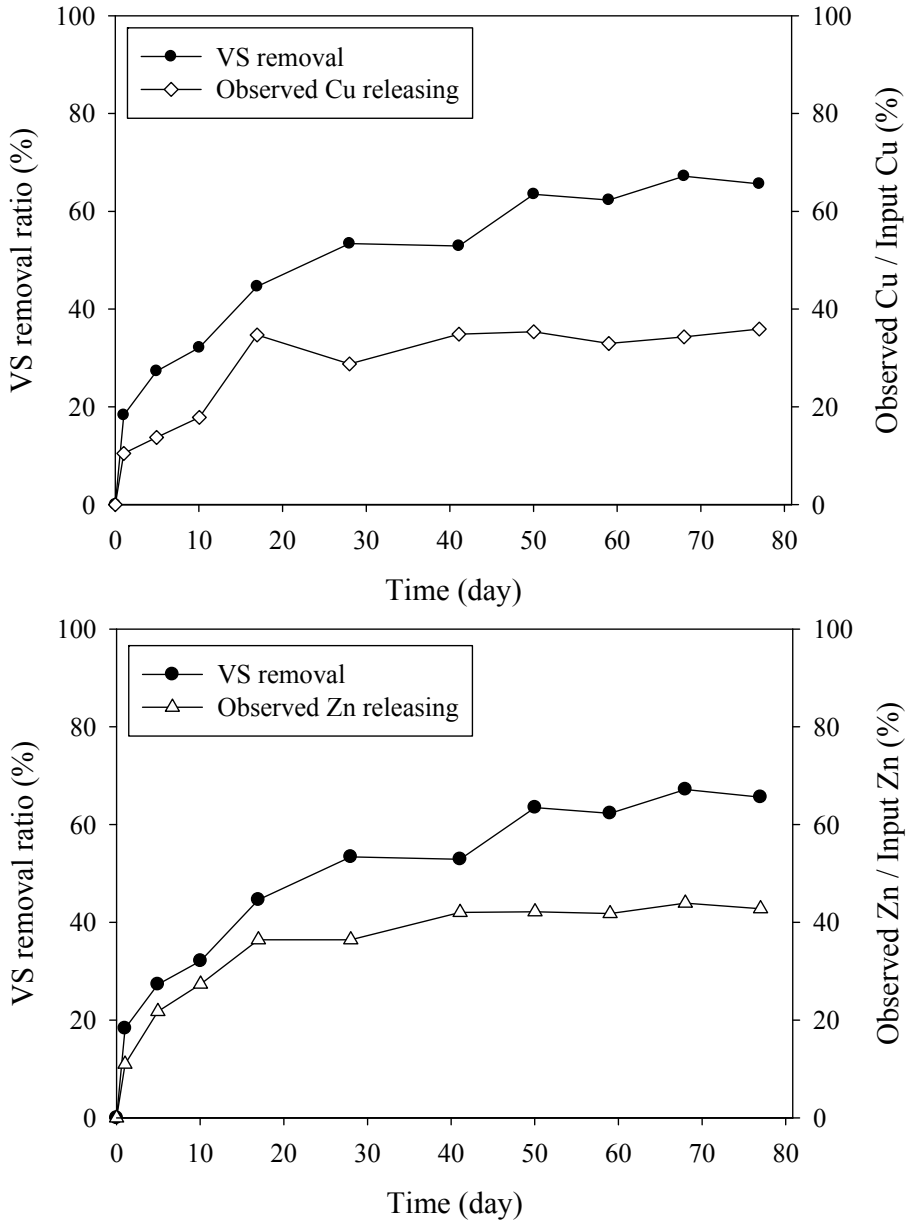

Figure 5.6 The soluble Cu and Zn ratio (observed mass/input mass) released from biomass versus VS removal ratio

Interestingly, these results demonstrated that the releasing tendency of heavy metals from biomass was not correspondent to biomass degradation. A maximum of 60% by wt. biodegradability of the biomass was observed in this study. This inconsistency between biodegradation ratio of biomass and release of endogenous heavy metal may have resulted from different fate of heavy metals in solution after release. In other words, there may have been other mechanisms involved in the releasing characteristics of heavy metals from biomass in addition to the degradation of biomass.

Anaerobic digestion system is biochemically very complex, so soluble heavy metals released from biomass through biodegradation of biomass alone may not fully describe the mechanism that contributes to the amounts of soluble heavy metals. Due to the complexity of the anaerobic system, heavy metals may be involved in many physico-chemical processes including precipitation as sulfide (except Cr) and hydroxides (Lawrence and McCarty, 1965; Mosey and Hughes, 1975) and adsorption to the solid fraction, either to biomass or to inert particulate matter (Shen et al., 1993; Shin et al., 1997) during digestion. These two reactions (i.e., precipitation and adsorption) occur very fast and reach equilibrium within a short time. Presence of soluble heavy metals can be affected by the fate of heavy metals after being released from biomass. Therefore, studies on determining the fate of heavy metals after being released from biomass should be carried out.

5.3.3 Major existing form of released heavy metals in solution (predicted by Visual Minteq 3.0)

From lab-scale anaerobic batch test results, it was emphasized that the presence of soluble released heavy metals from biomass was strongly dependent on the species of heavy metals. The prediction results of major existing form of heavy metals in solution using Visual Minteq 3.0 are shown in Table 5.2.

When release according to biomass degradation was assumed, most of the amount of Cd released from biomass was precipitated as complexation form of $Cd(HS)_2$. Likewise, released Pb was also precipitated mostly with sulfur and formed as $Pb(HS)_2$. Generally, Cd and Pb are classified into 'class B' (soft Lewis acids) by Pearson's classification (Pearson, 1963). Such classified heavy metals are known as sulfur seekers. Also, soft Lewis acids heavy metals, which mean hard Lewis bases, complex well with hydroxide. On the other hand, Cu, Ni, and Zn were not precipitated due to them having the opposite tendency of Cd and Pb. However, visual MINTEQ predicted that Cu, Ni, and Zn remained as ionic states in solution. The accurate percentage of total concentration of complexation form can be changed according to input parameters, but the trend of complexation was similar. According to Tyagi et al. (1988), the solubility of heavy metals can be affected by several factors such as pH, redox potential, and the concentrations of heavy metals and ligands (negative ions and uncharged molecules). Among these factors, previous research emphasized that the solubility of heavy metals is governed primarily by pH and redox potential. However,

pH and redox potential are barely changed in stable anaerobic digestion process and appropriate ranges for stable operation have been reported. Ultimately, the amounts of soluble heavy metals could be influenced by the concentrations of heavy metals and ligands. Usually abundant sulfur/sulfide compounds and hydroxide are contained in anaerobic digestion process, and some heavy metal species which complex well with sulfur and hydroxide (i.e., Cd, Pb) might be removed by sulfide or hydroxide precipitation.

Table 5.2 Results of predicted precipitation heavy metals amounts with sulfide and hydroxide

Heavy metal species	Complexation form	% of total concentration
Cd^{2+}	$Cd(HS)_2$	95.9
	$Cd(HS)^{3-}$	3.72
	$Cd(HS)_4^{2-}$	0.38
	Others ($Cd(OH)_2$, $Cd(OH)^{3-}$, $Cd(OH)_4^{2-}$, Cd^{2+}, Cd_2OH^{3+}, $CdHS^{+}$, $CdOH^{+}$)	0.01
Cu^{2+}	$Cu_2S_3^{2-}$	87.2
	CuS	11.1
	$CuOH^{+}$	0.58
	Cu^{2+}	1.11
	$Cu(OH)_2$	0.03
	Others ($Cu(OH)^{3-}$, $Cu(OH)_4^{2-}$, $Cu_2(OH)_2^{2-}$, Cu_2OH^{3+}, $Cu_3(OH)_4^{2+}$)	0.001
Pb^{2+}	$Pb(HS)_2$	98.6
	$Pb(HS)^{3-}$	1.40
	Others ($Pb(OH)_2$, $Pb(OH)^{3-}$, Pb^{2+}, Pb_2OH^{3+}, $Pb_3(OH)_4^{2+}$, $Pb_4(OH)_4^{4+}$, $PbOH^{+}$)	0.0000002

Ni^{2+}	$NiHS^{+}$	99.5
	Ni^{2+}	0.49
	Others ($Ni(OH)_2$, $Ni(OH)^{3-}$, $NiOH^{+}$)	0.001
Zn^{2+}	$Zn_2S_3^{2-}$	90.7
	ZnS	9.28
	$Zn_4S_6^{4-}$	0.01
	Zn^{2+}	0.04
	Others ($Zn(OH)_2$, $Zn(OH)^{3-}$, $Zn_2(OH)_4^{2-}$, Pb_2OH^{3+}, $ZnOH^{+}$)	0.001

5.3.4 Biosorption of heavy metals onto sorbents (differential binding affinity)

In the anaerobic digestion process, precipitation and adsorption reaction between soluble heavy metals and sorbents (i.e., sludge and crop residue) can occur at the same time. The soluble heavy metals released from crop residues can be adsorbed onto sorbents. The results of adsorbed heavy metals onto each sorbent are shown in Fig. 5.7.

Although different adsorption tendencies between heavy metal species and sorbent type were observed, Cu and Zn both showed the lowest adsorption amount in both cases. The differential amount of adsorbed heavy metals onto sorbent is probably due to their differential binding affinities. The binding affinity can be defined as the strength of interaction between heavy metals and sorbents (Davis et al., 2003; Murphy, 2007). The binding affinity can affect the amounts of soluble heavy metals released from biomass and it is dependent on the type of sorbents and the species of heavy metals. Cu, Ni, and Zn are known to have similar characteristics such as being on the border line of soft and hard metal, being transition metals, and having the same period on the periodic table. They are also known to have comparable molecular weight, atomic radius, and Vander Waals radius. However, Ni biosorption was not in accordance with this classification. As the result of adsorption test, Cu and Zn had relatively lower binding affinity than Ni to the specific sorbent.

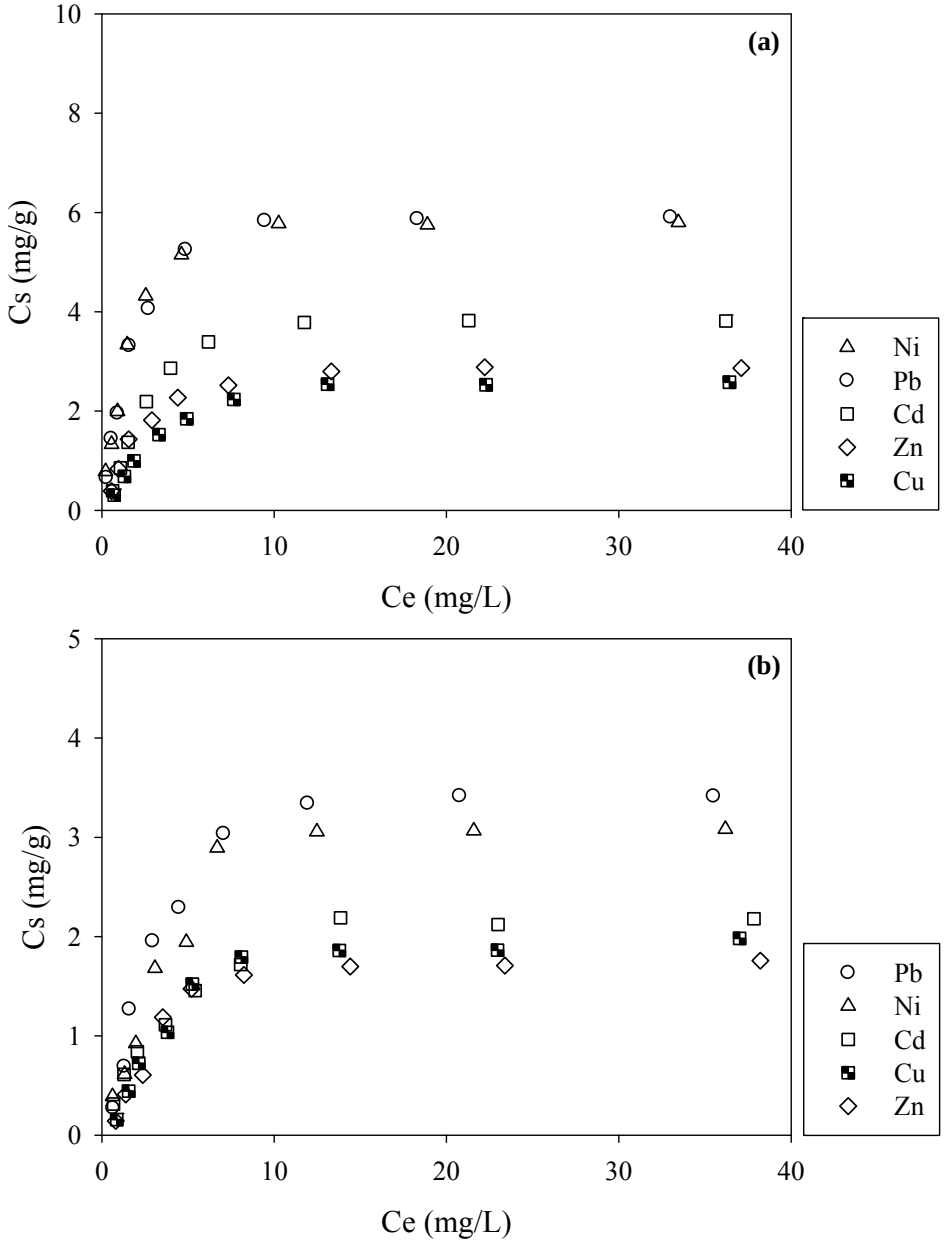

Figure 5.7 Adsorption isotherm of heavy metals (i.e., Cd, Cu, Ni, Pb, and Zn) with the sludge

Several researches also reported that Cu and Zn had lower binding affinities onto sorbents (i.e., sludge and crop residues) compared to other heavy metals such as Cd, Ni and Pb. Artola et al. (1997) reported that copper has lower binding capacity than other heavy metals (nickel and cadmium). Luo et al. (2006) reported that the sorption capacity of the sludge for Cd is preferential to Zn. Yuncu et al. (2006) also found that Cd had a higher biosorption capacity than Cu and Zn through sorption equilibrium tests using sludge as sorbent. Hammaini et al. (2007) reported that higher amount of Pb can be adsorbed onto activated sludge than Cu and Zn. Keskinkan et al. (2004) studied the adsorption characteristics of Cu, Pb and Zn on submerged aquatic plant *Myriophyllum spicatum*. The adsorption capacities were 10.37 mg/g for Cu, 46.69 mg/g for Pb and 15.59 mg/g for Zn. Sathasivam and Haris (2010) revealed that the adsorption affinity of heavy metal onto biomass (banana trunk fibers) can differ according to heavy metal species. They observed that Cd had a higher biomass-metal affinity compared to Cu and Zn. Tiemann et al. (1999) and Lezcano et al. (2011) also investigated that Pb had a greater biomass-metal affinity than Cu. The results of adsorption tests and the previous researches demonstrated that soluble Cu and Zn are hard to adsorb onto sorbents due to their lower binding affinity compared to Cd, Pb, and Ni. Although Cd, Pb, and Ni might have been released from crop residue, most of them could have been adsorbed onto biomass due to their strong binding affinities between heavy metals and biomass. Consequently, soluble Cd, Pb, and Ni were not observed in this study.

When multiple sorbents exist in heavy metal solution, competition of adsorption

can occur between sorbents and soluble heavy metals in solution. In the competition between crop residue and sludge as existing sorbents, most of released heavy metals from crop residue can be adsorbed onto sludge due to the relative abundance and faster reaction kinetics between the sludge and heavy metal. Thus, soluble heavy metals released from crop residues might be predominantly adsorbed onto sludge and very few amounts of heavy metals can be adsorbed onto crop residue. The adsorption results of sludge were both well fitted with Langmuir and Freundlich isotherm model ($R^2 >$ 0.90). Adsorption isotherms most commonly used to model the uptake of metals by the sludge biomass are the Langmuir and Freundlich isotherm (Brown and Lester, 1979). These two models have simpler structure for prediction of heavy metal adsorption compared with modified models in literatures. The Langmuir isotherm model was relatively better fit than the Freundlich isotherm model. The determination coefficients (R^2) and the obtained adsorption coefficients from adsorption test are shown in Table 5.3.

Table 5.3 Linear regression data for Langmuir and Freundlich isotherm for the adsorption of heavy metals (i.e., Cd, Cu, Ni, Pb, and Zn) with the sludge

Type of sorbent	Heavy metal species	Langmuir parameters			Freundlich parameters		
		Q_{max}	b	R^2	k	n	R^2
Sludge	Cd^{2+}	5.42	0.24	0.97	1.17	0.52	0.90
	Cu^{2+}	3.59	0.20	0.99	0.72	0.52	0.94
	Ni^{2+}	7.06	0.53	0.98	2.46	0.41	0.90
	Pb^{2+}	7.35	0.46	0.99	2.33	0.45	0.93
	Zn^{2+}	3.42	0.39	0.98	1.06	0.41	0.90

5.4 Summary

This study focused on the releasing characteristics of heavy metals from heavy metal-containing crop residues and the fate of heavy metals after release. Interestingly, the releasing tendency of heavy metals from crop residue was not correspondent to the degradation tendency of crop residue. Among Cd, Cu, Ni, Pb, and Zn, only Cu and Zn were observed in solution, most likely due to not only their different binding affinities between heavy metal species and biomass but also precipitation tendencies. The fate of heavy metals released from substrate via adsorption and precipitation were strongly influenced by heavy metal amounts in liquid phase. Although heavy metals in biomass are known as potential inhibitors in the anaerobic digestion process, not all heavy metals necessarily exist in ionized form within liquid phase of the system. Thus, the fate of heavy metals after release is the most significant factor for accurate prediction of the amounts and the adverse effects of released heavy metals from biomass on the performance of anaerobic digesters for heavy metal-containing biomass.

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CHAPTER 6
A MODEL DEVELOPMENT FOR PREDICTION OF HEAVY METAL CONCENTRATIONS WITH DEGRADATION OF CROP RESIDUES

6.1 Introduction

A model of the anaerobic digestion process which attempts to explain the complex patterns of the anaerobic digestion process is required to better understanding and design anaerobic digestion process. Mathematical models have provided an understanding of important inhibition patterns and have given guidelines for operation and optimization of anaerobic digesters (Angelidaki et al., 1999). For these purposes, a large number of mathematical models have been developed to simulate biodegradation of substrate, cell growth, and accumulation of input substances in anaerobic digestion system. The first dynamic mathematical models emerged in the late 1960's as an attempt to explain complex behavior of anaerobic reactors (Andrews, 1968; Graef and Andrews, 1974). The mathematical model developed in this study was primarily based on the general mass balance equation form of continuous stirred-tank reactor (CSTR). The mathematical model presented here describes the change of heavy metal concentrations in anaerobic digester according to degradation of heavy metal-containing biomass. Especially, it was focused on soluble heavy metal concentrations

which known to directly toxic to anaerobic microorganism. Among the various metal forms in anaerobic digestion process, only soluble heavy metals, free form are toxic to the microorganisms (Lawrence and McCarty, 1965; Mosey and Hughes, 1975; Oleszkiewicz and Sharma, 1990). Several studies have confirmed that the heavy metal toxicity correlated better to the metal's free ionic concentration (determined through a combination of dialysis and ion exchange) than to its total concentration (Bhattacharya et al., 1995). In addition to physico-chemical form, differences in substrate, bacteria genre, and environmental factors also explain the wide variation (from several to several hundreds of mg/L) in both the reported dosages of heavy metals and their relative toxicity (Bhattacharya et al., 1995; Hickey et al., 1989; Jin et al., 1998; Lawrence and McCarty, 1965; Lin and Chen, 1999; Zayed and Winter, 2000). When design an anaerobic digester for heavy metal-containing biomass, a mathematical model is needed not only for stable operation to prevent reactor failure attributed from the heavy metals in biomass but also for design of leachate and sludge final disposal method. Unfortunately, there are only few study of mathematical model for prediction of heavy metal concentrations in anaerobic digester according to biomass degradation.

The objective of this study was to develop and verify the adequate mathematical model based on the mass balance equation of CSTR. Furthermore, a sensitivity analysis was performed to illustrate the influence of variables (i.e., input parameters) such as organic loading rates, hydraulic retention times, kinetics of substrate degradation, and specific growth rates of microorganism.

6.2 Model development

A completely mixed system, or continuously stirred tank reactor (CSTR), is among the simplest systems that can be used to model a natural water body. It is appropriate for receiving water in which the contents are sufficiently well mixed as to be uniformly distributed.

The basics of CSTR can be written as following:

- Generally constant flow in and out.
- Contents are thoroughly mixed (Perfect mixing assumption).
- Concentration of a species in the effluent is equal to its concentration throughout the reactor.
- The analysis of a CSTR is based on (1) a mass balance on species within the fluid in the reactor accounting for processes, as well as (2) mass transport and (3) out of the reactor.

The general mass balance equation for CSTR with the previous mentioned basics can be expressed as follows:

$$\textbf{Accumulation rate} =$$

$$\textbf{Inflow rate} - \textbf{Outflow rate} \pm \textbf{Net transformation rate}$$

where, Net transformation rate: gain "+" and loss (or sink) "-"

Inflow rate $= Q_{in} \cdot C_{in}$

Outflow rate $= Q_{out} \cdot C_{out}$

Accumulation rate $= \frac{d(C \cdot V)}{dt}$

where, Q_{in}= flow rates of fluid in [L^3/T]

Q_{out}= flow rates of fluid out [L^3/T]

Q= flow rates of fluid [L^3/T]

C_{in}= concentration of the species in the inflow [M/L^3]

C_{out}= concentration of the species in the outflow [M/L^3]

C= concentration of the species [M/L^3]

V= volume [L^3]

T= elapsed time [T]

In this study, to predict the change of soluble heavy metal concentrations in CSTR according to biomass degradation, the mass balance model of CSTR was modified and applied. Fig. 6.1 shows the schematic drawing of heavy metal balance in CSTR. The main assumptions made to the model as follows:

(1) The substrate is a single biodegradable substance;

(2) The temperature [K] of CSTR is constant;

(3) The system volume [L^3] is constant.

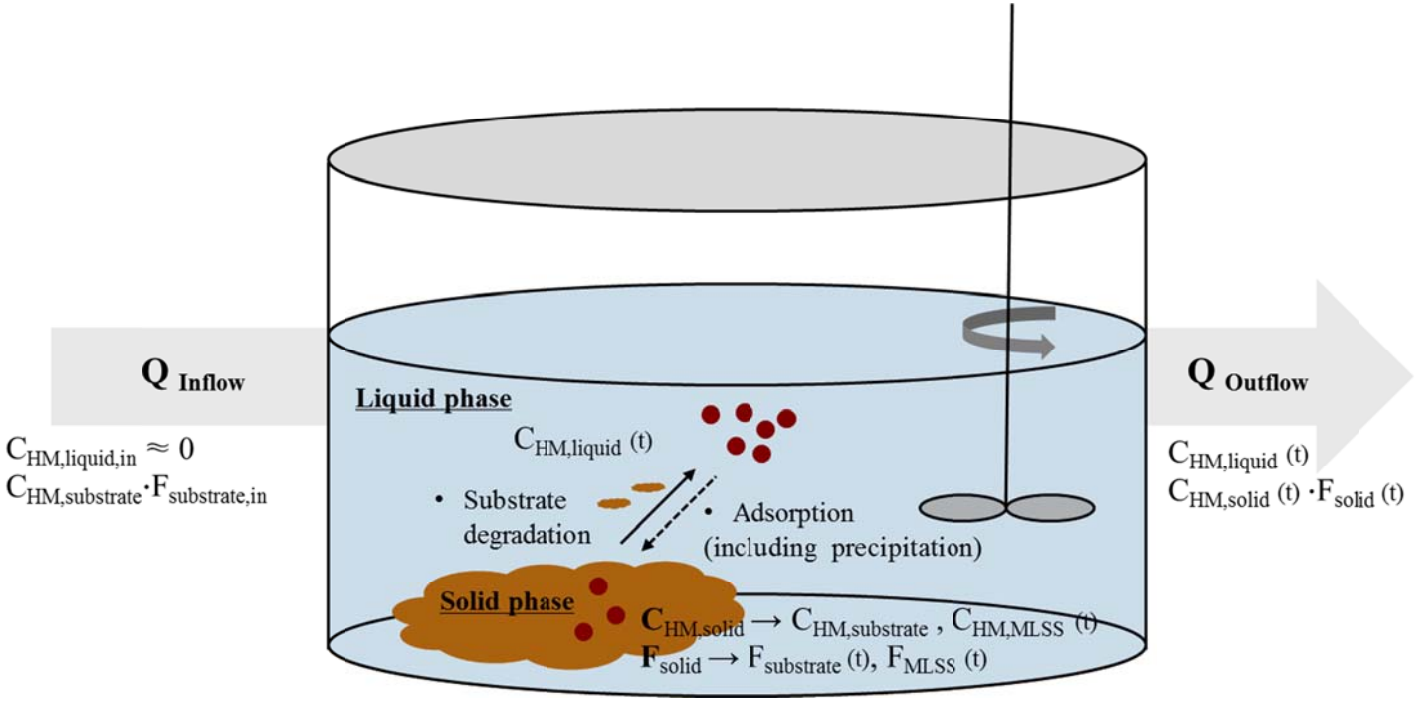

Figure 6.1 Schematic drawing of heavy metals mass balance in CSTR

Description of components used in the developed model and its unit in this study are explained in Table 6.1.

Table 6.1 Components used in the model developed in this model

Symbol	Description	Unit
V	System volume	L^3
Q	Flow rate	L^3/T
$C_{HM,l}$	Heavy metal concentration in water	M_{HM}/ L^3
$C_{HM,l,in}$	Heavy metal concentration in water (influent)	M_{HM}/ L^3
$C_{HM,MLSS}$	Heavy metal concentration in MLSS	M_{HM}/M_{MLSS}
$C_{HM,sub.}$	Heavy metal concentration in substrate (constant)	$M_{HM}/M_{substrate}$
$F_{sub.}$	Fraction of substrate in water	$M_{substrate}/ L^3$
$F_{sub.,in}$	Fraction of substrate in water (influent)	$M_{substrate}/ L^3$
F_{MLSS}	Fraction of MLSS in water	M_{MLSS}/ L^3
k	Kinetic of substrate degradation	1/T
Q_0	Maximum adsorption amount	M/M
K_L	Adsorption equilibrium constant	-
μ_{net}	Net specific growth rate	1/T

The heavy metals concentrations in liquid phase of CSTR can be represented as follows:

$$\boldsymbol{V}\frac{\boldsymbol{dC_{HM,l}(t)}}{\boldsymbol{dt}} = \boldsymbol{Q}\cdot\boldsymbol{C_{HM,l,in}} - \boldsymbol{Q}\cdot\boldsymbol{C_{HM,l}(t)} + \boldsymbol{V}\left\{\frac{\boldsymbol{dC_{HM,l}(t)}}{\boldsymbol{dt}}\Big|_{\boldsymbol{sub.release}} + \frac{\boldsymbol{dC_{HM,l}(t)}}{\boldsymbol{dt}}\Big|_{\boldsymbol{adsorption}}\right\} \quad \text{Eq. 6.1}$$

since V= constant

$$\frac{\boldsymbol{dC_{HM,l}(t)}}{\boldsymbol{dt}} = \frac{\boldsymbol{1}}{\boldsymbol{\theta}}\left(\boldsymbol{C_{HM,l,in}} - \boldsymbol{C_{HM,l}(t)}\right) + \left\{\frac{\boldsymbol{dC_{HM,l}(t)}}{\boldsymbol{dt}}\Big|_{\boldsymbol{sub.release}} + \frac{\boldsymbol{dC_{HM,l}(t)}}{\boldsymbol{dt}}\Big|_{\boldsymbol{adsorption}}\right\} \quad \text{Eq. 6.2}$$

where, $\theta = \frac{V}{Q}$

i) Heavy metal released from solids to water by substrate degradation

- assumption: substrate hydrolysis is modeled as 1st order reaction

$$\frac{dF_{sub.}(t)}{dt} = -k\cdot F_{sub.}(t) \quad \text{Eq. 6.3}$$

- assumption: heavy metal is released by hydrolysis of substrate

$$\left.\frac{dC_{HM,l}(t)}{dt}\right|_{sub.release} = C_{HM,sub.}\left(-\frac{dF_{sub.}(t)}{dt}\right) = k \cdot F_{sub.}(t) \cdot C_{HM,sub.} \qquad \text{Eq. 6.4}$$

ii) Heavy metal adsorption to solids

- assumption: there are only two reactions (i.e., adsorption and precipitation) occur in liquid phase of CSTR
- assumption: the amount of adsorbed heavy metal (obtained from the empirical results) includes the amount of precipitated heavy metal
- assumption: adsorption occurs only to MLSS

  (1) MLSS$\gg$Substrate in the reactor

  (2) Heavy metal has higher affinity to MLSS than to the substrate

$$\left.\frac{dC_{HM,l}(t)}{dt}\right|_{adsorption} = -\frac{d}{dt}\left(C_{HM,MLSS}(t) \cdot F_{MLSS}(t)\right) \qquad \text{Eq. 6.5}$$

- assumption: instantaneous equilibrium and adsorption follows Langmuir isotherm equation

$$C_{HM,MLSS}(t) = \frac{Q_0 \cdot K_L \cdot C_{HM,l}(t)}{1+K_L \cdot C_{HM,l}(t)} \quad \text{Eq. 6.6}$$

- MLSS change can be described as,

- assumption: μ_{net} is constant

$$\frac{dF_{MLSS}(t)}{dt} = \mu_{net} \cdot F_{MLSS}(t) \quad \text{Eq. 6.7}$$

- Overall heavy metal adsorption onto MLSS:

$$\frac{d}{dt}\left(C_{HM,MLSS}(t) \cdot F_{MLSS}(t)\right) = F_{MLSS}(t)\frac{dC_{HM,MLSS}(t)}{dt} + C_{HM,MLSS}(t)\frac{dF_{MLSS}(t)}{dt}$$

$$= F_{MLSS}(t)\frac{d}{dt}\left(\frac{Q_0 \cdot K_L \cdot C_{HM,l}(t)}{1+K_L \cdot C_{HM,l}(t)}\right) + C_{HM,MLSS}(t) \cdot \mu_{net} \cdot F_{MLSS}(t)$$

$$= F_{MLSS}(t) \cdot \frac{Q_0 \cdot K_L}{1+K_L \cdot C_{HM,l}(t)} \cdot \frac{dC_{HM,l}(t)}{dt} - \frac{Q_0 \cdot {K_L}^2 \cdot C_{HM,l}(t)}{\left(1+K_L \cdot C_{HM,l}(t)\right)^2} \cdot \frac{dC_{HM,l}(t)}{dt} + C_{HM,MLSS}(t) \cdot \mu_{net} \cdot$$

$$F_{MLSS}(t) \quad \text{Eq. 6.8}$$

⇒ Overall mass balance equation of heavy metal in liquid phase can be written as:

$$\frac{dC_{HM,l}(t)}{dt} = \frac{1}{\theta}\left(C_{HM,l,in} - C_{HM,l}(t)\right) + k \cdot F_{sub.}(t) \cdot C_{HM,sub.} - F_{MLSS}(t)\left(\frac{Q_0 \cdot K_L}{1+K_L \cdot C_{HM,l}(t)} - \frac{Q_0 \cdot K_L{}^2 \cdot C_{HM,l}(t)}{\left(1+K_L \cdot C_{HM,l}(t)\right)^2} + C_{HM,MLSS}(t) \cdot \mu_{net} \cdot F_{MLSS}(t)\right)\frac{dC_{HM,l}(t)}{dt} \quad \text{Eq. 6.9}$$

Mass balance equation can be simply re-written as:

$$\mathbf{R}\frac{dC_{HM,l}(t)}{dt} = \frac{1}{\theta}\left(C_{HM,l,in} - C_{HM,l}(t)\right) + k \cdot F_{sub.}(t) \cdot C_{HM,sub.} \quad \text{Eq. 6.10}$$

where, $R = 1 + F_{MLSS}(t)\left(\frac{Q_0 \cdot K_L}{1+K_L \cdot C_{HM,l}(t)} - \frac{Q_0 \cdot K_L{}^2 \cdot C_{HM,l}(t)}{\left(1+K_L \cdot C_{HM,l}(t)\right)^2} + \frac{Q_0 \cdot K_L \cdot C_{HM,l}(t)}{1+K_L \cdot C_{HM,l}(t)} \cdot \mu_{net}\right)$ Eq. 6.10.1

If adsorption is at linear range, then;

$$\rightarrow R = 1 + F_{MLSS}(t)\left(Q_0 \cdot K_L + Q_0 \cdot K_L \cdot C_{HM,l}(t) \cdot \mu_{net}\right) \quad \text{Eq. 6.10.2}$$

$$= 1 + F_{MLSS}(t) \cdot K_d\left(1 + \mu_{net} \cdot C_{HM,l}(t)\right) \quad \text{Eq. 6.10.3}$$

where, $K_d = Q_0 \cdot K_L$

6.3 Sensitivity analysis

The sensitivity analysis is performed to input variable affecting simulation results significantly. Under the identical simulation condition with input parameters fixed, a target variable was changed from -20 to +20% of its initial value. The simulation result using the initial value was considered as the reference, R. Finally, the sensitivity of a target variable was defined as follows:

$$\mathbf{SE}\ (\mathbf{i},\mathbf{n}) = \frac{\boldsymbol{R}\ (\boldsymbol{i},\boldsymbol{n}) - \boldsymbol{R_0}\ (\boldsymbol{i},\boldsymbol{n})}{\boldsymbol{R_0}\ (\boldsymbol{i},\boldsymbol{n})} \times \mathbf{100}\ (\%) \qquad \text{Eq. 6.11}$$

Where, i represents variables (OLR, HRT, k, μ_{net}). SE (i,n) is the sensitivity of variable i to the simulation result of n. This SE value has identical meaning with the error (%) of simulation result. R (i,n) is the simulation result of n with variable i at steady state, and R_0 is the reference value of n using the initial value of variable i.

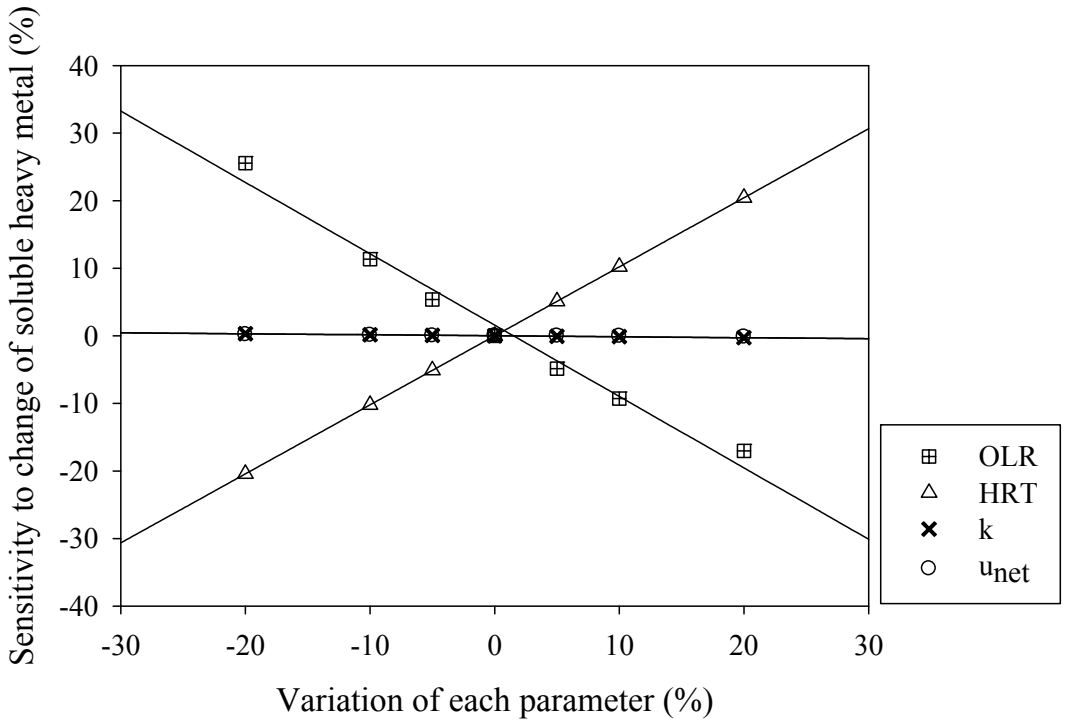

Figure 6.2 Sensitivity of variables to change of heavy metal concentrations in liquid phase (simulation HTR, OLR, and Time is 20 days, 1.5 g VS/L/day, and 200 days, respectively)

Fig. 6.2 illustrate the sensitivity of variables such as OLR, HRT, k, and μ_{net} to change of soluble heavy metal concentrations. From the result of sensitivity analysis, gradient of trend lines were obtained and used to compare sequence of sensitivity of variables (Table 6.2).

Table 6.2 Gradient of trend lines from sensitivity analysis

	Gradient of trend line
OLR	1.0565
HRT	1.0218
k	0.0146
μ_{net}	0.0090

The change of OLR has the highest sensitivity among the variables and HRT was comparable. This result is acceptable in common sense, because lower OLR means input low absolute amount of heavy metals to reactor and higher OLR refers to the opposite. And HRT also has the same meaning of OLR.

Although substrate degradation kinetic (k) has relatively low sensitivity to the change of heavy metal concentrations in liquid phase, k could be one of important input parameter due to its different characteristics depending on the type of substrate

and expanding the application of the model for various crop species. From Lehtomäki and Björnsson (2006) reported that degradation kinetic of crop residues under anaerobic condition might appear to vary from 0.010 to 0.090 day^{-1} according to their species. For reactor design and operating, other variables (i.e., OLR and HRT) could be adjusted depending on the circumstances, but degradation kinetic of crop residue is a dependent variable. Therefore, the change of heavy metal concentrations in CSTR could appear differently according to its degradation kinetic significantly.

6.4 Model verification and validation

6.4.1 Model verification

In order to evaluate the model reliability, the extreme situation in which the result is expected manifestly is simulated. Simulation conditions mainly conducted as follows: HRT=20 days, OLR=1.5 g VS/L/day, Simulation period=200 days. For verify the developed model, two extreme situations were assumed and simulated: Situation I for no substrate degradation in CSTR (no heavy metal releasing from crop residue); Situation II for no reactions of dissolved heavy metals in solution (no adsorption and precipitation).

6.4.1.1 Situation I: No substrate degradation in CSTR (no heavy metal releasing from crop residue)

In this case, the hydrolysis kinetic of substrate is zero. Considering no substrate degradation in CSTR ($k=0\ day^{-1}$), it can be surmised that there are no heavy metal releasing from substrate to liquid phase (Fig. 6.3). The 'Situation I' was compared with sunflower, which was used in the prior section of this study, degradation kinetic ($k=0\ day^{-1}$) as ordinary situation. The solid line and dotted line represent the ordinary situation and 'Situation I', respectively. The one of main assumption for developed model is that heavy metal releasing form substrate is based on the hydrolysis of substrate. Therefore, in the 'Situation I', no heavy metal releasing in reactor is acceptable result calculated from developed model.

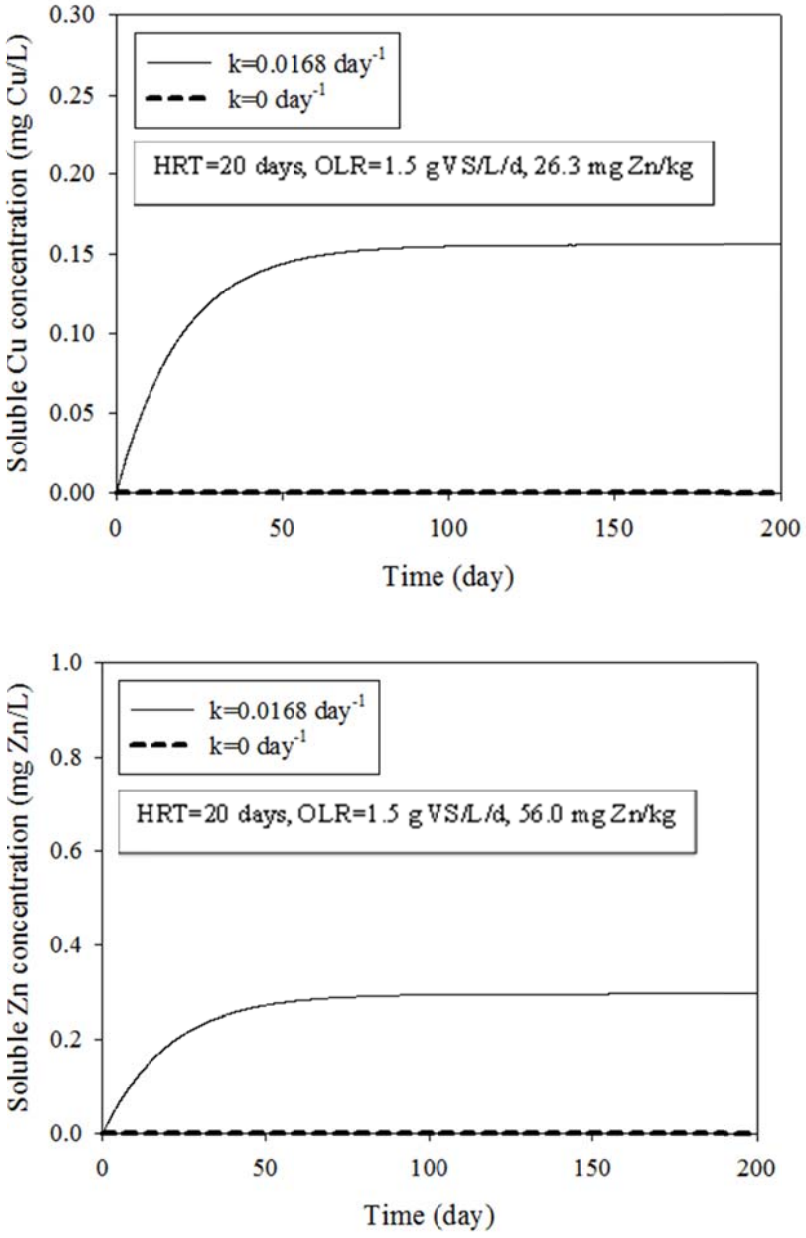

Figure 6.3 Simulation results of the condition of no substrate degradation

6.4.1.2 Situation II: No reactions of dissolved heavy metals in solution (no adsorption and precipitation)

The other case for no reactions of dissolved heavy metals in solution was simulated (Fig. 6.4). This implies that change of heavy metal concentrations in liquid phase follows only substrate degradation. For simulate 'Situation II', term of reaction (i.e., adsorption of released heavy metals onto MLSS) was input as zero. The 'Situation II' (without reaction) was compared with the ordinary situation (with reaction), observed result of adsorption reaction from the prior section of this study, adsorbed onto MLSS (including precipitation reaction) as ordinary situation. The solid line and dotted line represent the ordinary situation and 'Situation II', respectively.

The soluble concentrations of heavy metals in developed model is calculated from released amounts of heavy metals from substrate, amounts of heavy metals in effluent, and removed amounts of heavy metals from liquid phase by adsorption reaction. From the simulation results, the gap of soluble heavy metal concentration between with reaction and without reaction, there was only difference in amounts of heavy metals removed from liquid phase by adsorption reaction. Therefore, there is no diverge of the result for 'Situation II', no reactions of dissolved heavy metals in solution, calculated from developed model.

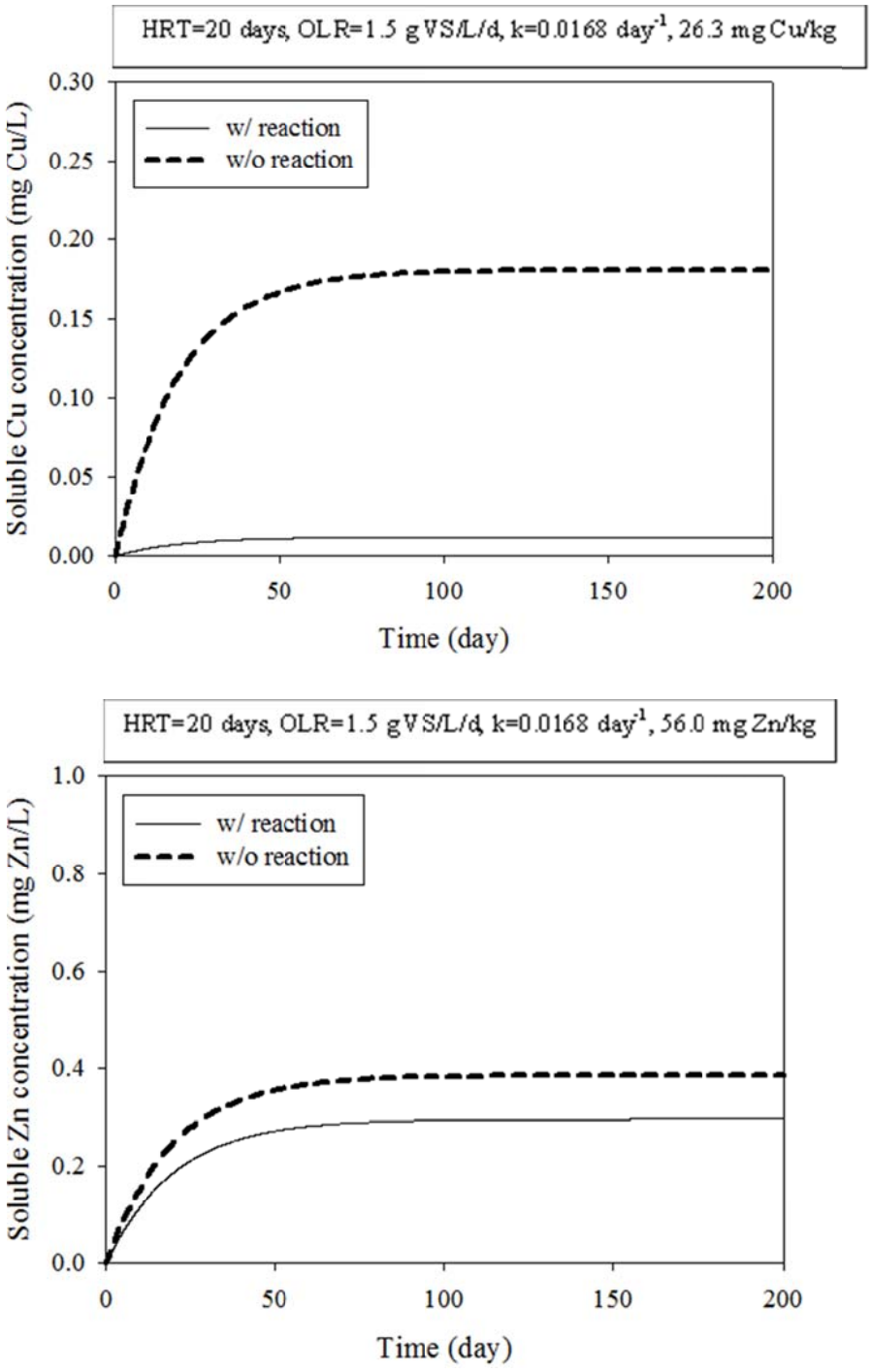

Figure 6.4 Simulation results of the condition of no adsorption and precipitation

6.4.2 Model validation

For the model validation, lab scale CSTR (1,080 days) are simulated, and then modeling results were compared to the observed data. Table 6.3 shows the conditions of operated CSTR which used to model verification.

Table 6.3 Model simulation and CSTR operating conditions

	Operating period (day)	HRT (days)	OLR (g VS/L/day)	$C_{HM,sub.}$ (mg $_{HM}$/kg substrate)	
				Cu	Zn
Phase I	95	30	1.0	26.3	56.0
Phase II	152	24	1.25	26.3	56.0
Phase III-1	653	20	1.5	26.3	56.0
Phase III-2	100	20	1.75	26.3	56.0
Phase III-3	80	20	2.0	26.3	56.0

Fig. 6.5 represents the simulation results of heavy metal concentrations change in CSTR. Until 240 days (during the Phase I and II), the model simulation could not describes well the observed data. However, after 300 days (during the Phase III), the model was relatively well fitted to observed data in within 20% significant level. In the early stage of observed data, dissolved heavy metal concentrations in reactor was unsettled due to the heavy metals come from inoculum. The inoculum sludge used in observed data was obtained municipal sewage treatment plant and it contained high concentrations of heavy metals. Although three months or a sufficient period of time

was taken before start operating reactor, it seems that inoculum destabilize the heavy metal concentrations in reactor. Looking at the trend of heavy metal concentrations in liquid, the heavy metal concentrations increased over time and reached a steady state at same HRT. As increasing of OLR with same HRT in next phase, similar trend was observed that the repeated. The model also well reflects trend of soluble heavy metal concentrations change in reactor during the Phase III.

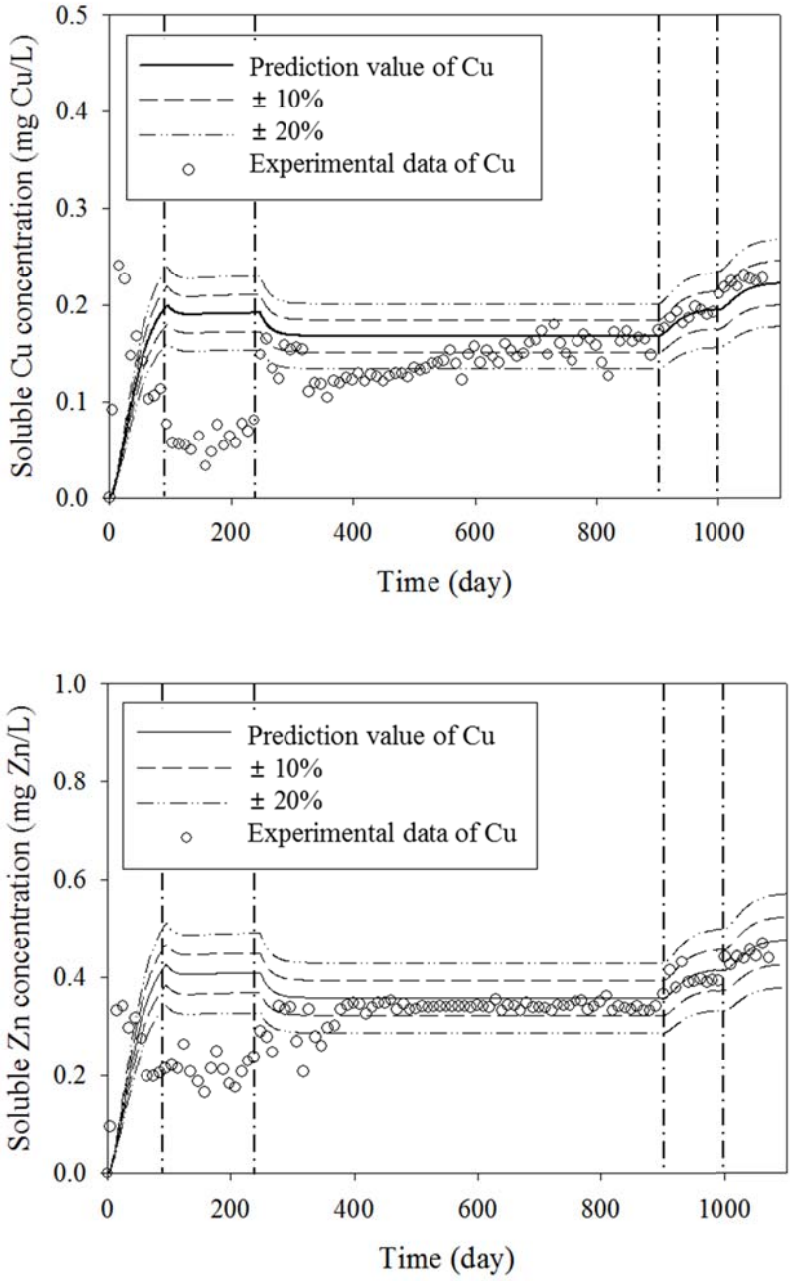

Figure 6.5 Simulation results of heavy metal concentrations change in CSTR

6.5 Application of developed model

The model developed in this study can predict the soluble heavy metal concentrations in anaerobic digestion process for heavy metal-containing substrate. Form the prediction results, operating parameters such as OLR and HRT can be designed and additional leachate treatment necessity can be determined. Operation of anaerobic digestion with the designed parameters will enable treatment of heavy metal-containing substrate without any heavy metal adverse effects. Furthermore, application of developed model for various substrates seems to be achieved using simple batch tests for substrate degradation kinetic and adsorption behavior. Among the constituents of developed model, substrate degradation kinetic and adsorption behavior of soluble heavy metal could be changed with type of substrate and reactor inert condition, respectively.

6.5.1 Maximum OLR for stable operation without inhibition of heavy metal

The example of soluble heavy metal concentrations in anaerobic digester for heavy metal-containing crop residue according to change of OLR is shown in Fig. 6.6. Simulation conditions were mainly conducted as following: HRT=20 days, k=0.0168 day^{-1}. The OLR was increased stepwise at every 200 day-period until the simulated soluble heavy metal concentration exceeded the reported inhibition level. From the simulation results, applicable OLR for copper and zinc was calculated equally around 10.0 g VS/L/day. OLR of 10.0 g VS/L/day is extremely high when compared to the OLR value of conventional anaerobic digester for crop residues. In general, previous researches suggest that effective anaerobic digestion performance can be maintained at a maximum OLR of 3 g VS/L/day with HRT near 20-25 days. Thus, heavy metal concentration levels of crop residues used in this study will not exert adverse effects to anaerobic bacterial activities under normal operating conditions.

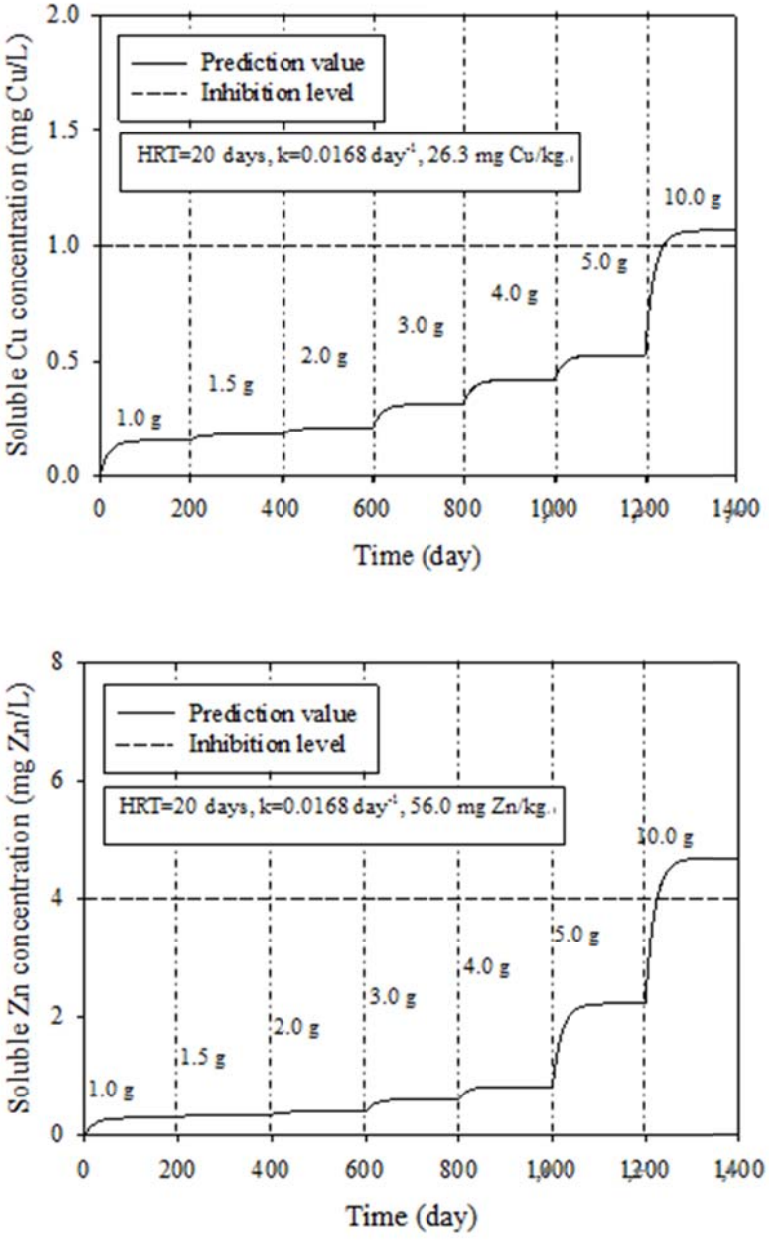

Figure 6.6 Example of soluble heavy metal concentrations in anaerobic digester for heavy metal-containing crop residue with change of OLR

6.5.2 Distribution of heavy metals between solid/liquid phase

When heavy metal-containing crop residues are treated by anaerobic digestion, the need for post treatment facilities can be determined by the model developed in this study. Distribution of heavy metals between liquid and solid phase was evaluated through assessment of relative contribution of releasing and adsorption term in model (Fig. 6.7-6.8). The simulation was conducted with following conditions: OLR of 1.5 g VS/L/day, HRT of 20 days, and k=0.0168 day^{-1}. The simulation result shows similar tendency to the test results in previous chapters.

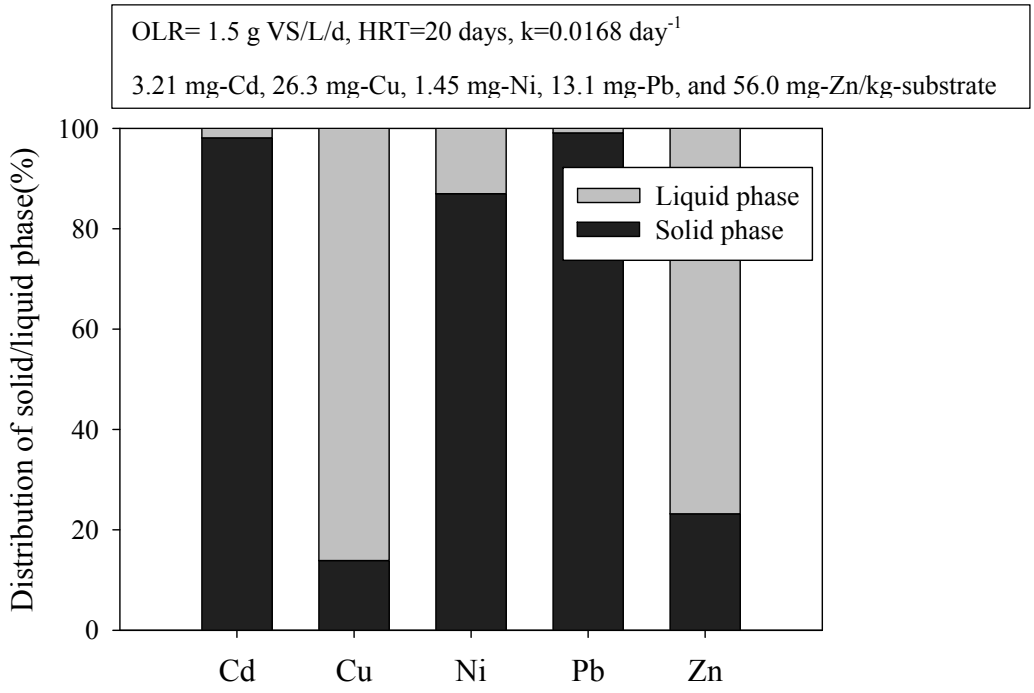

Figure 6.7 Example of heavy metal distribution in solid/liquid phase to determine the need for post treatment facility installation

As shown in Fig. 6.7, most of Cu and Zn exist in liquid phase, but on the contrary Cd, Ni, and Pb mainly exist in solid phase. If each concentration of heavy metal in solid and liquid phase exceeds allowable effluent standard through assessment of relative contribution of releasing and adsorption term in model, appropriate design for leachate and sludge final disposal method have to be combined. The necessity of waste-water treatment facility installation should be considered for Cu and Zn in leachate and the necessity of heavy metal recovery equipment should be considered for Cd, Ni, and Pb in sludge (Fig. 6.8).

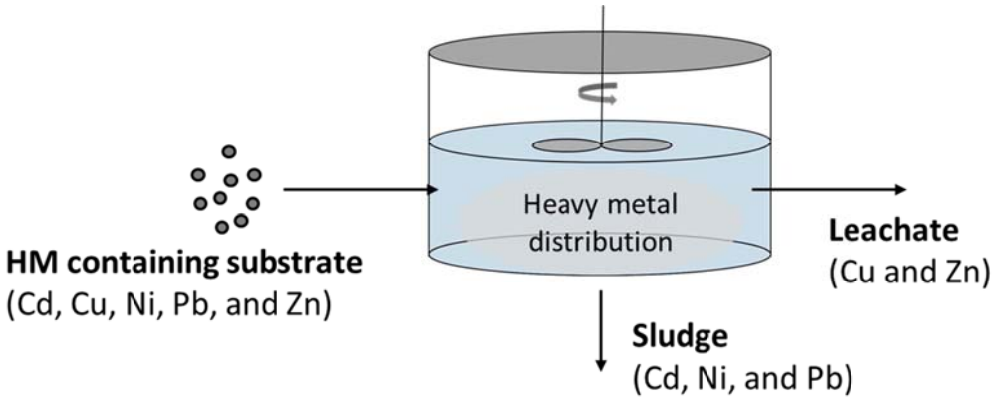

Figure 6.8 Example of predicted heavy metal distributions between leachate and sludge

6.5.3 Change of soluble heavy metal concentrations by substrate characteristics

The change of soluble heavy metal concentrations is dependent upon substrate characteristics (i.e., heavy metal concentration in substrate, kinetic of substrate degradation) (Fig. 6.9). When OLR and HRT are fixed for anaerobic digestion process design, heavy metal concentrations in process can be influenced by substrate characteristics. It seems that applicable heavy metal-containing crop residues can be classified with the developed model.

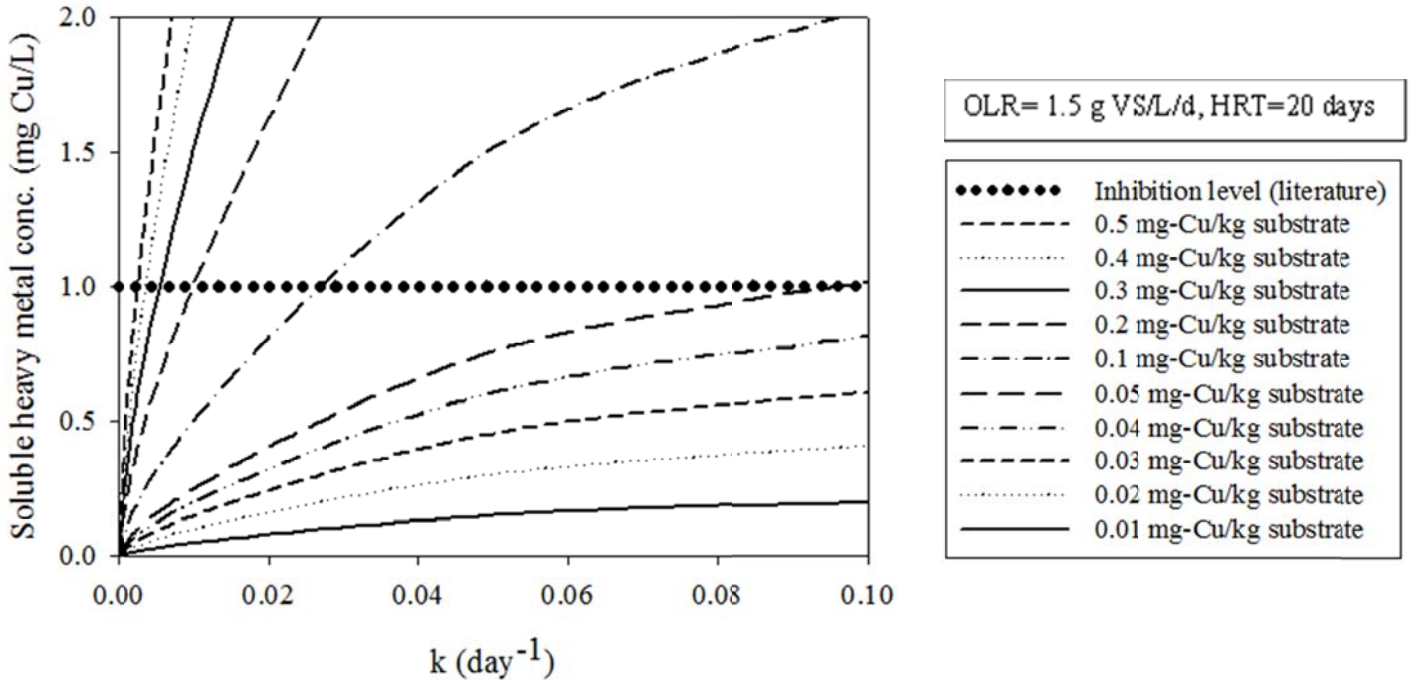

Figure 6.9 Change of soluble heavy metal concentrations by substrate characteristics

6.6 Summary

In this study, to simulate the change of soluble heavy metals in anaerobic digestion system, a mathematical model based on mass balance is developed. The model can describe the soluble heavy metal concentrations in anaerobic digester according to degradation of heavy metal-containing crop residues. From the sensitivity analysis for the variables used in the model, OLR has the highest sensitivity with gradient of trend line. Although substrate degradation kinetic (k) has relatively low sensitivity to the change of heavy metal concentrations in liquid phase, the k value was confirmed as the most important input parameter due to its variation with type of substrate. The developed model will provide useful information on anaerobic digestion process design for heavy metal-containing substrate and will expand the substrate types using simple batch test for substrate degradation kinetics. However, the model developed in this study includes several uncertain assumptions for the convenience of calculation (i.e., MLSS is constant during digestion, heavy metal adsorption occurs only to MLSS, etc.). Consequently, upgrading the developed model should be accompanied by verification and improvement of the uncertain assumptions for degree of completion.

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CHAPTER 7
CONCLUSIONS

The primary objective of this study is to suggest anaerobic digestion as a treatment method for crop residues from heavy metal contaminated sites. Detailed conclusions are given at the end of each chapters. The general conclusions referring to the three specific objectives proposed at the beginning of this study are presented herein.

(1) Although the crop residues contained differential amounts of heavy metals, including maximum level of heavy metal for normal growth of sunflower, there was no significant difference in methane gas production among crop residues. Furthermore, during the laboratory scale CSTR operation periods, adverse effects of heavy metals on reactor performance was not observed (i.e., biogas production, methane content in biogas, organic matter decomposition, VFAs concentration, alkalinity, and pH) and microbial communities, commonly found in anaerobic digestion process for cellulosic biomass, established stably with respect to the substrate. Therefore, when accompanied with a proper design of practical operation parameters, anaerobic digestion of heavy metal-containing crop residues from phytoremediation sites can be an appropriate approach without adverse effects of heavy metals.

(2) The releasing tendency of heavy metals from crop residues was not correspondent to the degradation tendency of crop residues. Among various heavy metal species (i.e., Cd, Cu, Ni, Pb, and Zn), only Cu and Zn were observed in solutions. This result is likely due to not only their different binding affinities between heavy metal species and biomass but also their precipitation tendencies. The fate, such as adsorption and precipitation, of heavy metals released from substrate strongly influence to the ultimate heavy metal amounts in liquid phase. Thus, the fate of heavy metals after release is the most significant factor for accurate prediction of the amount and the effects of released heavy metals from biomass on the performance of anaerobic digestion process.

(3) A mathematical model for the prediction of heavy metal concentrations in anaerobic digestion process for heavy metal-containing biomass was developed. The model describes the soluble heavy metal concentrations in an anaerobic digester according to degradation of heavy metal-containing crop residues. The developed model will provide useful information on anaerobic digestion process design for heavy metal-containing biomass and will expand the substrate types using simple batch test for substrate degradation kinetics. However, the model developed in this study includes several uncertain assumptions for the convenience of calculation (i.e., MLSS is constant during

digestion, heavy metal adsorption occurs only to MLSS, etc.). Consequently, upgrading the developed model should be accompanied by verification and improvement of the uncertain assumptions for degree of completion.

국문초록

중금속 함유 식물체부산물의 처리를 위한 혐기성소화에 대한 연구

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중금속 오염토양의 식물상정화공법 이후 발생되는 식물체부산물은 식물의 생장 과정에서 토양 내 존재하는 중금속을 흡수하고 이를 체내에 축적한 상태로 수확된다. 따라서 식물상정화공법 이후 발생되는 식물체부산물은 적절한 방법을 통해 처리되어야 한다. 최근 식물체부산물의 혐기성소화를 통한 바이오가스 생산에 대한 연구가 많은 연구자들에 의해 관심을 받고 있으며, 이와 관련하여 바이오가스 생산을 위한 바이오매스의 재배를 중금속 오염토양에서 실시할 경우 식물의 생장 과정 중 중금속 흡수를 통한 토양의 정화를 실시할 수 있을 뿐만 아니라 수확된 식물체부산물은 바이오가스 생산을 위한 연료로 사용될 수 있는 장점이 있다. 중금속 오염토양에서 수확된 식물체부산물 내 포함되어 있는

중금속은 혐기성소화 공정 내에서 혐기성 미생물의 대사와 활동에 영향을 미쳐 공정 자체의 실패를 초래하는 요인이 될 수 있다. 본 연구에서는 중금속 오염토양에서 수확된 해바라기부산물을 대상으로 중금속 함유 식물체부산물의 처리에 대한 혐기성소화 적용 타당성을 검증하였다. 또한 연속식 반응조의 물질수지를 바탕으로 혐기성소화 공정 내 중금속의 거동특성을 반영하는 중금속 농도 예측 모형의 개발을 실시하였다.

서로 다른 농도의 중금속 농도로 오염된 토양으로부터 수확된 해바라기부산물을 대상으로 BMP (Biochemical methane potential) test를 실시하여 최대메탄발생량을 측정하고 이를 비교함으로써 중금속을 함유하는 식물체부산물의 혐기성소화를 통한 처리 가능성을 평가하였다. 그 결과 비오염토양에서 수확된 해바라기부산물부터 해바라기가 정상적으로 생장할 수 있는 최고 수준의 중금속 농도로 오염된 토양에서 수확된 해바라기부산물에 이르기까지 총 4종의 각기 다른 중금속 농도를 함유하는 해바라기부산물 간의 최대메탄발생량에서 유의미한 차이는 관찰되지 않았다 (201.60±11.39 - 227.38±15.59 mL CH_4/g VS). 이는 실험 종료 후 확인된 혐기성 미생물의 활동에 직접적으로 영향을 줄 수 있는 액상 내 중금속의 양이 대조군과 실험군들에서 유사한 수준으로 존재하였으며, 모두 문헌에서 제시하고 있는 저해 수준 이하였기 때문으로 판단된다. 결국 해바라기가 정상적으로 생장할 수 있는 수준의 중금속 오염토양에서 수확된

해바라기부산물은 중금속에 의한 영향이 없이 혐기성소화를 통한 처리가 가능할 수 있음을 의미한다.

실험실 규모의 연속식 완전혼합반응조 (CSTR)를 중온의 혐기성 조건에서 약 1,100 일 동안 운전함으로써 중금속을 함유하는 식물체부산물의 혐기성소화에 대한 공정의 안정성을 장기간에 걸쳐 평가하였다. 실험에는 폐광산 인근의 중금속 오염토양에서 실제 식물상정화공법을 실시하고 수확된 해바라기부산물을 사용하였다. 반응조 운전 결과, 비오염토양에서 수확된 일반 식물체부산물을 대상으로 혐기성소화를 실시했던 기존의 선행연구와 유사한 유기물부하량 (OLR) 2.0 g VS/L/day 및 수리학적체류시간 (HRT) 20일의 조건까지 반응조 액상 내 중금속 농도는 혐기성 미생물의 활동에 저해를 줄 수 있다고 보고되는 농도 이하로 유지되었다. 반응조의 운전 안정성을 평가할 수 있는 지표인 바이오가스 발생량, 바이오가스 내 메탄함량, 유기물제거율, 지방산 농도, 알칼리도, 그리고 pH 등에 대한 분석을 실시하였다. 분석 결과 확인된 지표들은 모두 반응조 운전 기간에 걸쳐 큰 변화를 보이지 않고 안정적인 범위를 유지됨에 따라 반응조가 외부로부터 유입된 독성물질에 의한 영향을 받지 않고 안정적으로 운전이 이루어졌음을 알 수 있었다. 또한 반응조 운전 기간 중 반응조 내 미생물 군집을 함께 확인한 결과, 대부분 셀룰로오스계 바이오매스의 혐기성소화조에서 관찰되는 미생물들이 시간의

흐름에 따라 기질에 순응하며 유사한 군집을 형성해 나아감을 확인하였다. 반응조 운전 후반부에 이르러 반응조 운전 초기에 비해 메탄생성균 (methanogen) 중 유기산의 축적과 관련된 *Methanosarcina* 속 (genus)이 점차 증가하였고, 이는 지속적인 유기물부하량의 증가와 수리학적체류시간의 감소로 인한 영향이 나타날 수 있음을 의미한다. 결국 중금속을 함유하는 식물체부산물의 혐기성소화 공정은 비오염토양에서 수확된 일반 식물체부산물과 같은 조건에서 부산물 내에 포함된 중금속의 영향을 받지 않고 안정적으로 운전될 수 있다. 다만 반응조 내 유기산의 축적을 피할 수 있는 적절한 운전조건 (i.e., 유기물부하량, 수리학적체류시간)에 대한 연구가 추후 수반되어야 할 것으로 판단된다.

본 연구에 사용된 해바라기부산물에는 구리, 납, 니켈, 아연, 그리고 카드뮴이 주요 중금속으로 포함되어 있었다. 그러나 해바라기부산물의 혐기성 분해가 이뤄진 이후 혐기성 미생물의 활동에 직접적으로 영향을 미칠 수 있는 액상 내 존재하는 형태의 중금속은 오직 구리와 아연만이 관찰되었다. 해바라기부산물의 분해와 부산물의 분해로 인해 액상 내에 존재하는 중금속의 양을 살펴보면 중온 혐기성소화 조건에서 부산물은 약 50일에 걸쳐 최초 투입된 양의 60% (휘발성고형물 중량비)가 분해되었지만 구리와 아연은 최초 투입된 양의 40% (총 중량비)가 부산물로부터 빠져나와 최종적으로 액상 내에 존재하는 것으로 확인되었다. 이는 중금속의

혐기성소화조 내 거동 특성을 통하여 설명될 수 있으며, 이온 형태의 중금속은 중금속별 특성에 따라 매우 복잡한 시스템 특성을 갖는 혐기성소화조 내에서 침전 또는 흡착을 통해 액상 내에서 제거될 수 있다. 해바라기 부산물 내에 포함되어 있는 개별 중금속의 특성을 살펴보면 '피어슨의 분류 (Pearson's classification)'에 따라 납과 카드뮴은 황 (sulfur) 또는 수산화이온 (OH^-)과 잘 결합될 수 있어 침전을 통해 액상에서 제거될 수 있으며, 실제 Visual MINTEQ를 이용한 중금속 이온의 존재형태 예측 결과에서도 대부분이 $Pb(HS)_2$ 와 $Cd(HS)_2$ 의 형태로 존재하는 것을 확인하였다. 또한 침전이 잘 이루어지지 않는 구리, 니켈, 아연 중 니켈은 흡착실험 결과 슬러지와 해바라기부산물 모두에 높은 결합 정도 (binding affinity)를 갖는 것이 확인되었다. 결국 식물체부산물 내에 포함된 모든 중금속은 혐기성소화에 영향을 미칠 수 있는 잠재적인 영향인자이나, 부산물의 분해에 따라 부산물로부터 빠져 나온 이후 이온상태로 존재할 때의 거동 특성에 따라 일부 중금속만이 최종적으로 혐기성 미생물의 활동에 영향 줄 수 있을 것으로 판단된다.

마지막으로 이러한 중금속의 거동 특성을 반영하며 연속식반응조의 물질수지를 바탕으로 하는 혐기성소화조 내 중금속 농도 예측 모형의 개발을 실시하였다. 개발된 모형 내 변수들에 대한 민감도 분석과 모형을 통한 계산이 정상적으로 이루어지는지 여부를 확인하기 위한 검증

(verification) 과정을 수행하였다. 최종적으로는 개발된 예측 모형을 이용한 계산값과 앞선 장의 연속식반응조 운전 결과 중 실측된 중금속 농도의 비교를 통한 모형의 유효성확인 (validation)을 실시하였다. 계산값과 실측값의 비교 결과 모형은 ±20% 오차범위 수준에서 혐기성소화조 내 중금속 농도 변화 경향을 비교적 잘 예측하는 것으로 나타났다. 개발된 모형을 이용하여 기질의 분해속도상수 (k)와 부산물 내 중금속 농도에 따른 반응조 내 중금속 농도를 표현하는 중금속 함유 식물체부산물의 혐기성소화조 설계 가이드를 제공할 수 있을 것으로 사료된다. 다만 본 연구에서 개발된 모형은 계산의 편리성을 위한 불확실성을 내재하는 가정들을 일부 포함하고 있기 때문에 이러한 가정의 검증과 개선을 통해 모형의 예측 결과에 대한 정확성을 제고할 필요가 있을 것으로 판단된다.

주요어: 혐기성소화; 중금속 함유 식물체부산물; 식물체부산물 처리; 기질 분해; 중금속 거동 특성, 중금속 농도 예측 모델 개발

학 번: 2011-20995