國 立 交 通 大 學

環境工程研究所

碩 士 論 文

藉由溫相式厭氧消化 (TPAD) 系統將農牧廢棄物轉化製造生質肥料

Biofertilizer production from agriculture and livestock wastes by temperature-phased anaerobic digestion (TPAD)

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碩 士 論 文

A Thesis
Submitted to Institute of Environmental Engineering
College of Engineering
National Chiao Tung University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in

Environmental Engineering

August 2012

Hsinchu, Taiwan, Republic of China

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摘要

本研究結合溫相式厭氧消化(TPAD)及厭氧共消化之概念來處理農牧廢棄物(豬糞和稻稈)，並探討 TPAD 系統之效能、厭氧生物產能和製備生物肥料等目的。豬糞和稻稈在臺灣為主要的農牧廢棄物，且污染量和強度相對於其他農牧廢棄物高。一般而言，單一廢棄物經厭氧消化處理常有反應槽效能不佳或是微生物抑制問題產生，導致厭氧處理在應用上受限；消化二種或二種以上不同來源之廢棄物，有效提高厭氧效能並減少操作問題產生。

TPAD 系統是由前段高溫反應槽及後段中溫反應槽所組成，藉由高溫段提升整體系統之處理效能如揮發性固體物(VS)去除、產生大量生物沼氣及致病菌消滅，而中溫階段則負責洗鍊高溫出流，提升 TPAD 出流水品質及強化整體系統之穩定性。由於本研究在實驗設備上的問題導致 TPAD 系統在整個馴養期間受到相當大的影響，本研究之最大揮發性固體物濃度控制在 20 g VS/L，為避免阻塞問題發生。

擬穩態階段之數據顯示二個 PM 及 RS 比例(PM:RS=80:20 和 90:10)皆可達到 Class A biosolids 對於 VS 和致病菌的規範；進料大部分有機氮被轉化為氨氮，

然而消化後污泥中有機磷略微增加。從重金屬結果得知，本實驗之二比例在銅和鋅二金屬濃度遠高於其他金屬，且超出臺灣對液態肥料之標準，此外鉻和鎳也有超出規範的可能，這表示豬糞和稻稈比例在本實驗中仍未達到最佳比例。

關鍵字：溫相式厭氧消化(TPAD)、厭氧共消化、豬糞、稻稈、生物肥料

Biofertilizer production from agriculture and livestock wastes by temperature-phased anaerobic digestion (TPAD)

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Abstract

This study combined with temperature-phased anaerobic digestion (TPAD) and the concept of anaerobic co-digestion to treat agriculture and live stock wastes (pig manure and rice straw) and investigated the performances of TPAD system, anaerobic bioenergy production as well as biofertilizer production. Pig manure (PM) and rice straw (RS) are the main this type waste in Taiwan and the amount and strength compared to other agro-wastes are much high. In general, single source waste treated with anaerobic digestion often has poor reactor performances or microbial inhibition problems and results in a limitation of anaerobic treatment; co-digestion with two or more different sources wastes can effectively improve anaerobic performances and reduce operational problems.

TPAD system, which includes the first thermophilic stage and the second mesophilic stage reactors, takes the thermophilic stage to improve the system performance, volatile solid (VS) removal, producing a large amount of biogas as well

as pathogens elimination; while the mesophilic stage is responsible for polishing thermophilc effluent and strengthening the stability of whole system. Because the problem of laboratory equipments in this study caused a considerable impact in overall accumulation periods, the maximum VS concentration was 20 g VS/L in this research to avoid occurring obstruction problem.

The data of pseudo-steady-state conditions showed that two ratios of PM and RS (PM:RS=80:20 and 90:10) could meet the Class A biosolids for the specifications of the VS removal and pathogens reduction. Organic nitrogen in the substrate was converted to ammonium, however organic phosphorus in the effluent sludge slightly increased after digesting. From the result of heavy metals, the concentrations of copper and zinc were much higher than other metals and exceeded Taiwanese standards for liquid fertilizers, moreover the concentrations of chromium and nickel were also likely to exceed the standards, indicating both the ratios of PM and RS in this study didn't yet reach the optimum ratio.

Keywords: Temperature-phased anaerobic digestion (TPAD), Anaerobic co-digestion, Pig manure, Rice straw, Biofertilizer

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Chapter 1 Introduction

1.1 Background

People attach gradually importance to environment, water and energy issues with the increase in population. The energy requirement mainly relies on the use of fossil fuels nowadays, resulting in a large number of greenhouse gas emissions. Global warming and global climate change have become urgent crises, and significant amount of pollutants produced by human activities is also the main reason contributing to water pollution and shortage. A lot of agriculture and animal livestock wastes have been also the main reason causing environmental health and water pollution, and pig manure is the main problem of livestock wastes in Taiwan. Taiwanese pig farms usually use a three-stage treatment for piggery wastewater, which includes solid-liquid separation by screening method, anaerobic treatment and aerobic treatment (Tsai and Lin, 2009), and the solid part of swine manure is treated by using composting or landfill disposal. Rice straw, for example, is also main agriculture waste in Taiwan, usually treated with open burning or used as a compost material. Composting is the common method to treat high solid organic wastes, but still has some risks like incomplete elimination of pathogens (Nicholson et al., 2005), incomplete maturity or ammonia emission resulting in nitrogen loss as well as greenhouse gas emission. However another method, which has been developed more than a century and has a considerable potential to treat high solid wastes is the anaerobic biotechnology.

Anaerobic biotechnology is quite important in environmental engineering not only on the batter processing performance than aerobic biotechnology but on the producing useful by-products. In addition to anaerobic digestion (AD), which is

known by many people, there are also many novel anaerobic biotechnologies such as anaerobic fermentation to producing organic acids and anaerobic hydrogen production have been noticed in recent years (Chu et al., 2008; Khanal, 2008), however these biotechnologies still have some key issues to be solved. The application of conventional anaerobic digestion, for example, is unable to spread than aerobic treatment as the accumulation of methanogens is difficult and time-consuming. However, high-rate AD began to flourish since 1950 and caused AD could be applied to the high concentration of wastewater or organic wastes processing. Biogas production has improved significantly due to a high strength of wastes, therefore AD shifts from being just one part of the processing unit to becoming a biogas plant to produce bioenergy.

Temperature-phased anaerobic digestion (TPAD), which is composed of thermophilic and mesophilic two reactors is one of high-rate AD. In addition, waste or wastewater treated by thermophilic stage could effectively achieve pathogens eradication and obviously improve the availability of effluent to make as a biofertilizer or a soil conditioner. The concept of co-digestion is an emphasis on AD field in recent years, through two or more different sources wastes adjusted to appropriate concentration can significantly enhance the performances of AD process and overcome the inhibition problems occurring in anaerobic microbial metabolism.

Therefore, this study uses TPAD technique combining with the concept of co-digestion to treat pig manure (PM) and rice straw (RS) and to produce by-products like biogas and biofertilizer, and investigates the appropriate ratio of PM and RS to get the best fertilizer quality.

1.2 Objective

The objective of this study is using temperature-phased anaerobic digestion (TPAD) to treat rice straw (RS) and pig manure (PM) and evaluate the feasibility which takes TPAD effluent as a biofertilizer. We focused on the operation of microorganisms in TPAD system as well as reactors performances such as VS removal, biogas production and reduction of pathogens. Besides, the ratios of PM and RS, which were according to individual VS concentrations were also concerned in order to carry out the optimal TPAD operation condition, the optimal nutrient ratios of fertilizers as well as reducing the harmful ingredients like heavy metals.

Chapter 2 Literature review

2.1 Anaerobic processes: multi-step metabolism processes

When organic matters are decomposed by microorganisms at a condition without dissolved oxygen or its precursors (e.g. H_2O_2), these biological processes are known as anaerobic processes and can be further distinguished into anaerobic fermentation and anaerobic respiration, like Fig. 2-1.

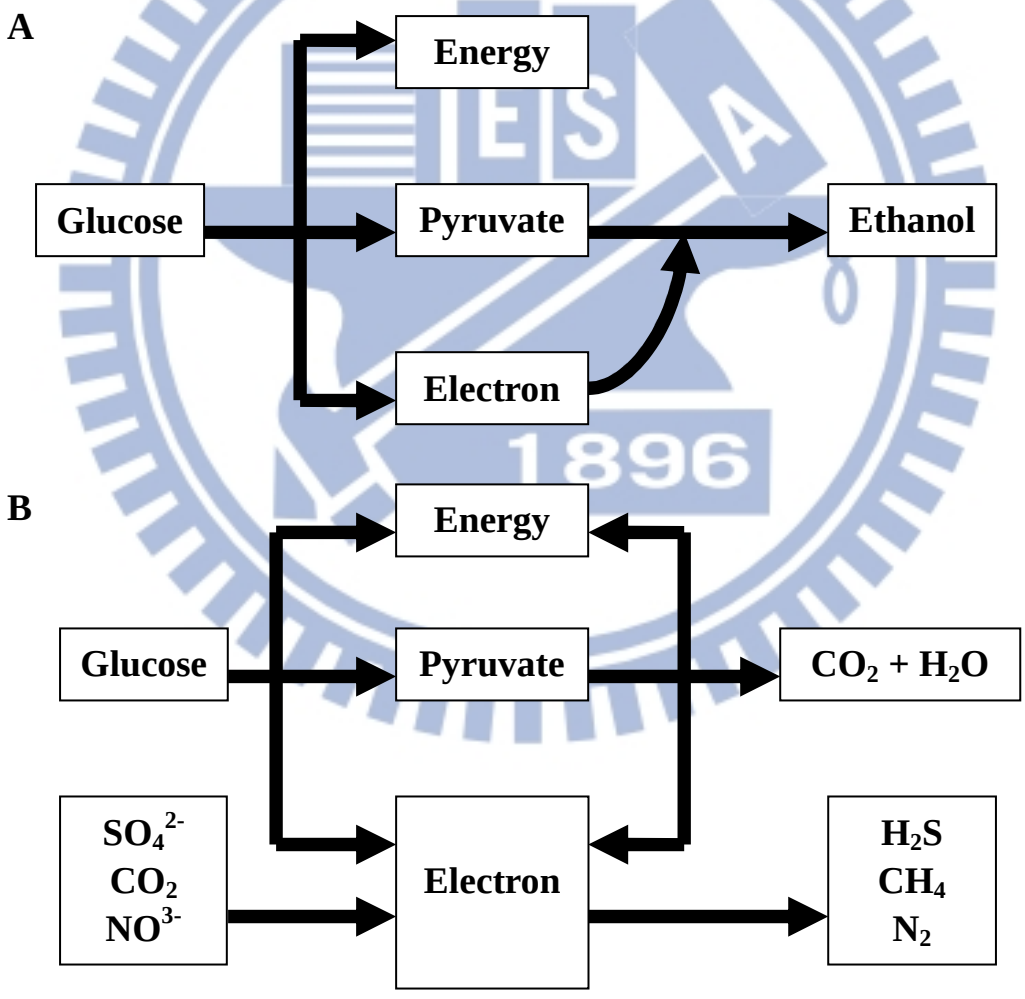


Fig. 2-1 (A) Anaerobic fermentation and (B) anaerobic respiration of glucose (Khanal, 2008).

Anaerobic fermentation means organic matter is catabolized in an absence of external electron acceptors by strict or facultative anaerobes via internally balanced oxidation-reduction reactions, on the other hand, anaerobic respiration, also called anaerobic digestion (AD), requires external electron acceptors for the disposal of electrons released during degradation of organic matter (Khanal, 2008).

2.1.1 Microorganisms and metabolism

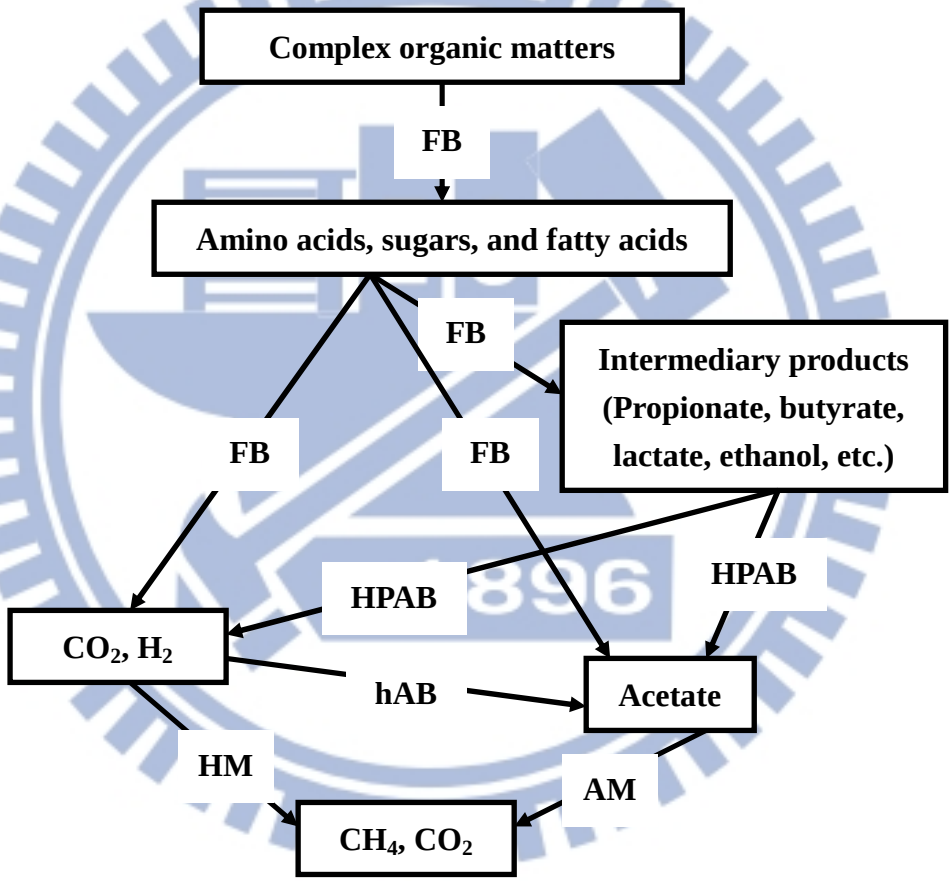


Fig. 2-2 The conversion pathways and microorganisms in anaerobic digestion (McCarty, 1964a; Bryant, 1979; 賀延齡, 1998; Demirel and Scherer, 2008; Khanal, 2008).
FB Fermentative bacteria; HPAB Hydrogen-producing acetogenic bacteria;
hAB Homoacetogenic bacteria; AM Acetotrophic methanogens;
HM Hydrogenotrophic methanogens

Fig. 2-2 is the conversion pathways in AD. We can see the first step in AD is

decomposing complex organic matters to simple small molecules, like amino acids, sugars and fatty acids, and then these simple molecules are further broken and become intermediary products, like propionate, butyrate, lactate and ethanol etc., via hydrolytic and fermentative bacteria. The second step is cleavage of these intermediary products to generate acetate or hydrogen and carbon dioxide through hydrogen-producing acetogenic bacteria, there have some special bacteria, which are known as homoacetogenic bacteria can transform hydrogen and carbon dioxide into acetate thus may compete with hydrogenotrophic methanogens. And the final step in anaerobic processes is the biomethanation which indicates both acetotrophic methanogens and hydrogenotrophic methanogens convert acetate, H_2 and CO_2 into CH_4.

2.1.1.1 Fermentative bacteria, H_2-producing acetogenic bacteria, and Homoacetogens

We have known the first step in anaerobic processes is hydrolysis and fermentation to produce intermediary products which are utilized by methanogens. So extracellular enzymes produced from hydrolytic and fermentative bacteria in this stage play an important role, and extracellular enzymes whether they can effectively decompose complex organic matters depend on the size of contact area between bacteria and substrates. In addition to enzymes, temperature, retention time, composition and particle size of organic matters, pH, ammonium concentration and hydrolyzate concentration (e.g. volatile fatty acids, VFAs) are parameters that can also affect hydrolysis rates significantly (賀延齡, 1998). Bacteria in this stage most belong to the family of streptococcaceae and enterobacteriaceae, such as *Bacteroides*, *Clostridium*, *Butyrivibrio*, *Eubacterium*, *Bifidobacterium* and *Lactobacillus*. Although these group bacteria can survive in obligate anaerobic condition, some of

them are facultative anaerobic organism which can bear existing low concentration of dissolved oxygen.

Fig. 2-3 is the pathways of carbohydrate fermentation, in this scheme polysaccharide is first degraded to sugar, and then sugar fermentation occurs mainly via the Embden-Meyerhof-Parnas pathway (EMP pathway) and transforms into pyruvate. Different final products generating from metabolism of pyruvate are dependent on different metabolic pathways, one way is pyruvate catalysis to yield acetate, butyrate, ethanol, CO_2 and H_2, and the other way is pyruvate catalysis via lactate or succinate metabolic pathways to generate propionate (Bryant, 1979). Hydrogen concentration is a key role in these processes because it makes a significant impact on acetate production even at very low H_2 level in the reactor. According to literatures, the propionate oxidation to acetate becomes thermodynamically favorable only at H_2 partial pressures below 10^{-4} atm, and for butyrate and ethanol oxidation below 10^{-3} and 1 atm, respectively (Khanal, 2008).

There have some researches about hydrolytic and fermentative phenomena in mesophilic or thermophilic AD. Liu et al. (2009) verified co-digestion of garbage and manure had a positive effect in thermophilic (53℃) AD performances even at the percentage of garbage in the mixed wastes was low (2-3%). Besides, bacterial species in the phylum *Firmicutes* were dominant bacteria responsible for the digestion of these wastes. Kim et al. (2010) investigated influence of high temperature (51℃) on bacterial community using mesophilic sludge inoculum. Denaturing gradient gel electrophoresis (DGGE) profiles shows the monitored bacterial community consisted of *Pseudomonas mendocina*, *Bacillus halodurans*, *Clostridium hastiforme*, *Gracilibacter thermotolerans*, and *Thermomonas haemolytica*. The function of *B. halodurans*, *G. thermotolerans*, and *T. haemolytica* are reported to carbohydrate

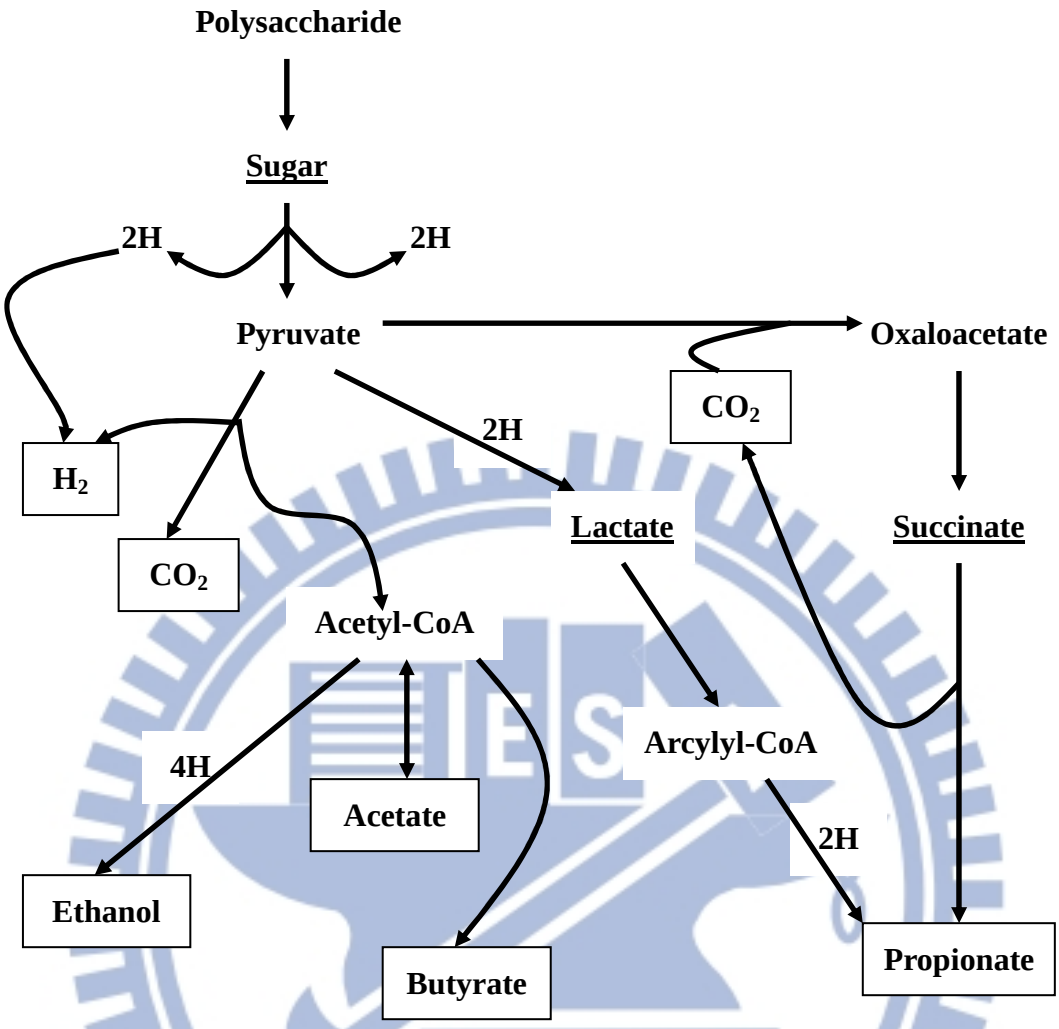


Fig. 2-3 Pathways involved in carbohydrate fermentation by hydrolytic and fermentative bacteria (Bryant, 1979).
□: Final product; __: Extracellular intermediate

fermentation thermotolerantly. In contrast, *P. mendocina* disappeared when temperature rose due to its mesophilicity. In addition, *C. hastiforme* and *G. thermotolerans* originating in mesophilic sludge but were also detected in the thermal acidogenesis. Above-mentioned bacteria, *B. halodurans*, *C. hastiforme*, and *G. thermotolerans* belong to Firmicutes, *P. mendocina* and *T. haemolytica* belong to γ-Proteobacteria.

The microbial community dynamics of a three stages mesophilc AD process,

which co-digestion with organic fraction of municipal solid waste (OFMSW) and fruit and vegetable waste was investigated by Supaphol et al. (2011). They found bacterial community was mainly constituted by Firmicutes, Actinobacteria, β-, γ-, and ε- Actinobacteria. These bacteria are responsible for producing VFAs, some of them also have functions such as denitrification, H_2S oxidation, or Fe reduction. Martín-González et al. (2011) studied the microbial community monitoring from a sewage treatment plant, which is a thermophilic (55℃) co-digestion of OFMSW and lipid-rich wastes and they found the composition of microbial community were major by Firmicutes, Bacteroidetes, Synergistes, and Thermotogae.

H_2-producing acetogenic bacteria are responsible for converting intermediary products into acetate, H_2 and CO_2 which are precursors for methanation. But from the following formulas (Eq. 2.1-2.4), we can see the Gibb's free energy changes of anaerobic oxidation of propionate, butyrate, benzoate, and ethanol are positive, in other words, this indicates anaerobes must consume energy to make reactions go to produce acetate. Fig. 2-4 is the photos about the anaerobic granule and the syntrophic relationship between H_2-producing acetogenic bacteria and methanogens.

Propionate → acetate

$$\mathbf{CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + H^+ + HCO_3^- + 3H_2}$$

$$\mathbf{\Delta G^\circ = +76.1\ kJ/reaction} \qquad \textbf{(Eq. 2.1)}$$

Butyrate → acetate

$$\mathbf{CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + H^+ + 2H_2}$$

$$\mathbf{\Delta G^\circ = +48.1\ kJ/reaction} \qquad \textbf{(Eq. 2.2)}$$

Benzoate → acetate

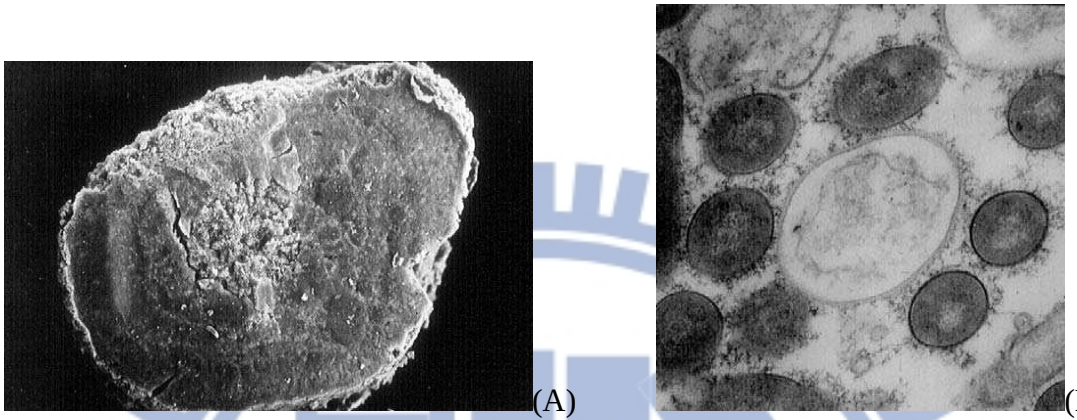
$$\mathbf{C_7H_5CO_2^- + 7H_2O \rightarrow 3CH_3COO^- + 3H^+ + HCO_3^- + 3H_2}$$

$$\mathbf{\Delta G^\circ = +53.0\ kJ/reaction} \qquad \textbf{(Eq. 2.3)}$$

Ethanol → acetate

$$CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^+ + 2H_2$$

$$\Delta G^\circ = +9.6 \text{ kJ/reaction} \qquad \text{(Eq. 2.4)}$$



(A) (B)

Fig. 2-4 (A) Scanning electron microscope image of a granule from an upflow anaerobic sludge bed reactor (UASB) (B) Transmission electron microscope image of an ultrathin section of a granule from an UASB-reactor (The images were provided by Grotenhuis, de Bok et al., 2004)

Temperature has a significant influence on thermodynamics because high temperature can reduce the amounts of free energy which are the demands for oxidizing propionate, butyrate, and ethanol. The following formulas (Eq. 2.5-2.10) are about oxidation of propionate in anaerobic processes. Apart from temperature, if methanogens rapidly scavenge H_2 or acetate producing from oxidation of intermediates and keep the level of H_2 partial pressure extremely low, this phenomenon is commonly known as "interspecies hydrogen transfer." Interspecies formate transfer may be also important in this symbiotic system since the diffusion distance of formate is than of hydrogen in water (賀延齡, 1998; de Bok et al., 2004).

$$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 3H_2$$

$$\Delta G^\circ = +76.1 \text{ kJ/mol (25℃)}; \quad \Delta G^\circ = +62.3 \text{ kJ/mol (55℃)} \qquad \text{(Eq. 2.5)}$$

$CH_3CH_2COO^- + 2HCO_3^- \rightarrow CH_3COO^- + H^+ + 3HCOO^-$

$\Delta G°$ = ＋72.2 kJ/mol (25℃); $\Delta G°$ = ＋59.7 kJ/mol (55℃) **(Eq. 2.6)**

$H_2 + 0.25HCO_3^- + 0.25H^+ \rightarrow 0.25CH_4 + 0.75H_2O$

$\Delta G°$ = －33.9 kJ/mol (25℃); $\Delta G°$ = －30.6 kJ/mol (55℃) **(Eq. 2.7)**

$HCOO^- + 0.25H_2O + 0.25H^+ \rightarrow 0.25CH_4 + 0.75HCO_3^-$

$\Delta G°$ = －32.6 kJ/mol (25℃); $\Delta G°$ = －29.7 kJ/mol (55℃) **(Eq. 2.8)**

$CH_3COO^- + H_2O + 0.25H^+ \rightarrow CH_4 + HCO_3^-$

$\Delta G°$ = －31.0 kJ/mol (25℃); $\Delta G°$ = －34.7 kJ/mol (55℃) **(Eq. 2.9)**

$CH_3CH_2COO^- + H_2O \rightarrow 1.75CH_4 + 1.75HCO_3^- + 0.25H^+$

$\Delta G°$ = －56.4 kJ/mol (25℃); $\Delta G°$ = －65.0 kJ/mol (55℃) **(Eq. 2.10)**

de Bok et al. (2004) summarized five conclusions concerning with interspecies electron transfer in propionate degradation. First, propionate oxidation requires obligate syntrophic consortia of acetogenic and H_2 and bicarbonate reducing methanogens. Second, the amount of energy released from the complete oxidation of propionate (under methanation conditions) is 1 ATP (about 60 kJ/mol), which has to be shared for three different organisms. Third, the majority of propionate-oxidizing bacteria oxidize propionate via the methyl-malonyl-CoA pathway yielding acetate, CO_2, H_2 or formate. But another pathway may occur, which is condensed to a six-carbon intermediate, and then this intermediate is cleaved to butyrate and acetate. Fourth, H_2 is an important interspecies electron transfer, but formate may be even more significant. Finally, aggregated biomass has a high conversion rates due to the small interbacterial distances.

Homoacetogens can utilize H_2 and CO_2, which are intermediary products produced from hydrolytic and fermentative bacteria to synthesize their final product, acetate, and these microorganisms are either autotrophs or heterotrophs. *Clostridium*

aceticum and *Acetobacterium Woodii* are the two mesophilic homoacetogenic bacteria isolated from sewage sludge. Homoacetogens may have a competitive relationship with hydrogenotrophic methanogens due to hydrogen which can be as an electron donor not only for homoacetogenic bacteria but also for hydrogenotrophic methanogens (Khanal, 2008). The following formulas (Eq. 2.11-2.12) can tell us this competition. However, more researches are needed to understand the interaction of these microorganisms in anaerobic processes.

$$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$$

$$\Delta G^\circ = -135.6 \text{ kJ/reaction} \quad \textbf{(Eq. 2.11)}$$

$$4H_2 + 2HCO_3^- + H^+ \rightarrow CH_3COO^- + 4H_2O$$

$$\Delta G^\circ = -104.6 \text{ kJ/reaction} \quad \textbf{(Eq. 2.12)}$$

2.1.1.2 Methanogens

We all know anaerobic processes are accomplished by consortia, which relate to hydrolytic and fermentative bacteria, acetogens and methanogens. Methanogens play a central role in the whole anaerobic processes, and there have three reasons: first, methanogens belong to the Archaea, and their physiologies and structures are distinct from bacteria. Archaea are unlike true bacteria due to presence of membrane lipids, absence of basic cellular characteristics (e.g., peptidoglycan), and distinctive ribosomal RNA (Rittmann and McCarty, 2001; Khanal, 2008). Second, compared with other anaerobes, methanogens grow slowly. If existence of a large amount of inhibitors in anaerobic processes, it will harm methanogens and reduce the processing performance of AD. And third, various modes of operation will affect the community composition of methanogens, for example, hydrogenotrophic methanogens are dominant in thermophilic AD, but the major methanogens in the

mesophilic AD are acetotrophic methanogens. In addition, unsuitable retention time will resulting in methanogens too late to grow due to washing out. Although both hydrogenotrophic and acetotrophic methanogens are responsible for producing methane, there are still many differences between them.

Methanogens that are responsible for producing methane are usually classified acetotrophic and hydrogenotrophic methanogens according to their biomethanation precursors. Moreover, the hydrogenotrophic conversion contributes up to 28% of the methane production, on the other hand, the acetotrophic conversion is responsible for surplus 72% of the methane production (McCarty, 1964a; Khanal, 2008). Tab. 2-1 is a classification of methanogens, we can find three classes methanogens including methanobacteria, methanococci and methanomicrobia, respectively. H_2 and CO_2, acetate and formate are important substrates for methanogenic bacteria to produce methane. Formate concentration is low than other substrates due to it is rapidly produced and consumed. All species, especially hydrogenotrophic methanogens, can use H_2 as an elector acceptor to reduce CO_2 to methane, these bacteria can synthesize methane by formate as well as H_2 and CO_2. But H_2 and CO_2 aren't only approach to accomplish biomethanation, acetate cleaves methane and bicarbonate is common reaction in AD. Although methanogens using acetate as the substrate are few, they still play a key role in anaerobic reactor since major biomethanation occurs via this way. *Methanosaricina* species are known to use acetate as the substrate, they often exist in a reactor which has high acetate concentration (Bryant, 1979; Demirel and Scherer, 2008; Khanal, 2008).

In regard to hydrogenotrophic methanogens, these bacteria utilize not only H_2 as elector donors reducing CO_2, buy also formate to produce methane, besides, these processes carry out either directly or indirectly. Although acetate, formate and H_2 and CO_2 are common substratres for methanogens, some of them can also oxidize

Tab. 2-1 Classification of methanogenic bacteria (adapt from Demirel and Scherer, 2008)

Class I. Methanobacteria (substrate: H_2/CO_2, carbon source: formate)
- Order I. Methanobacteriales
  - Family I. Methanobacteriaceae
    - Genus I. *Methanobacterium*
    - Genus II. *Methanobrevibacter*
    - Genus III. *Methanosphaera*
    - Genus IV. *Methanothermobacter*
  - Family II. Methanothermaceae
    - Genus I. *Methanothermus*

Class II. Methanococci (substrate: H_2/CO_2, carbon source: formate)
- Order I. Methanococcales
  - Family I. Methanococcaceae
    - Genus I. *Methanococcus*
    - Genus II. *Methanothermococcus*
  - Family II. Methanocaldococcaceae
    - Genus I. *Methanocaldococcus*
    - Genus II. *Methanotorris*

Class III. Methanomicrobia (substrate: H_2/CO_2, carbon source: formate)
- Order I. Methanomirobiales
  - Family I. Methanomicrobiaceae
    - Genus I. *Methanomicrobium*
    - Genus II. *Methanoculleus*
    - Genus III. *Methanofollis*
    - Genus IV. *Methanogenium*
    - Genus V. *Methanolacinia*
    - Genus VI. *Methanoplanus*
  - Family II. Methanocorpusculaceae
    - Genus I. *Methanocorpusculum*
  - Family III. Methanospirillaceae **(known to be hydrogenotrophic)**
    - Genus I. *Methanospirillum*
- Order II. Methanosarcinales **(known to be acetate- and methylotrophic)**
  - Family I. Methanosarcinaceae
    - Genus I. *Methanosarcina*
    - Genus II. *Methanococcoides*

Tab. 2-1 Continued on next page

Tab. 2-1 Continued

Genus III. *Methanofollis*
Genus IV. *Methanogenium*
Genus V. *Methanolacinia*
Genus VI. *Methanoplanus*
Family II. Methanocorpusculaceae
Genus I. *Methanocorpusculum*
Family III. Methanospirillaceae **(known to be hydrogenotrophic)**
Genus I. *Methanospirillum*
Order II. Methanosarcinales **(known to be acetate- and methylotrophic)**
Family I. Methanosarcinaceae
Genus I. *Methanosarcina*
Genus II. *Methanococcoides*
Genus III. *Methanohalobium*
Genus IV. *Methanohalophilus*
Genus V. *Methanolobus*
Genus VI. *Methanomethylovorans*
Genus VII. *Methanimicrococcus*
Genus VIII. *Methanosalsum*
Family II. Methanosaetaceae
Genus I. *Methanosaeta*

compounds that contain methyl groups, mono-, di-, and trimethylamine, and dimethyl sulfide in terms of literatures (賀延齡, 1998; Khanal, 2008).

Different operation of AD can obviously change the community that is a composition of methanogens as well as acetogenic bacteria. Thermophilic condition or operating at short retention time can favor rod like or coccoid hydrogenotrophic methanogens. In addition, *Methanosaeta* spp. are the dominant aceticlastic methanogen at low acetate concentration, but they decrease fast when the acetate concentration increases. Contrarily, high acetate concentration is accompanied an increase in *Methanosarcina* spp. (Demirel and Scherer, 2008).

Mesophilic operation is a more common method then thermophilic operation in AD treatment, but thermophilic methanogens have still attracted much attention in

recently due to their resistance to extreme environment and potentialities on Environmental Engineering. Tab. 2-2 is Suryawanshi et al. (2010) listed new species thermophilic methanogens found in recent years from many previous studies, these archaea were found in wildly varying habitats.

Operation modes certainly change the both community of fermentative anaerobes and methanogens. Liu et al. (2002) examined the start-up of two acidogenic reactors under mesophilic (37℃) and thermophilic (55℃) conditions carried out with methanogenic granular sludge as an inoculum and dairy wastewater as feed. From the DGGE results, due to pH drop to 5.5, the domains *Bacteria* and *Archaea* populations showed significant shifts after 13 days operation accompanied with an increase in VFAs production, a decrease in methane formation, and rapid sludge disintegration. *Methanosaeta* were abundant at the interior of the seed sludge and many other biogranules, but the dominant population changed from *Methanosaeta* to *Methanomicrobiales*, *Methanobacteriales* and *Methanococcales* when reactors were operated at a high VFAs concentration and a low pH condition, besides, the microbial community change was more significant and rapid in the thermophilic reactor. Although obvious community changes took place at the first 13 days for both reactors, a longer period up to 71 days was required to make the microbial community more stable.

The methanogens population change in the study of Liu et al. (2009) was found *Methanoculleus* (hydrogenotrophic) and *Methanosarcina* (acetotrophic) were responsible for methane generation in thermophilic upflow anaerobic filter reactor. Sasaki et al. (2011) carried out their experiment with thermophilic (55℃) AD using artificial garbage slurry as feed. In addition, they also took a tracer experiment using ^{13}C-labeled acetate and found approximately 80% of the acetate was decomposed via a non-aceticlastic oxidative pathway (Eq. 2.14), whereas the remainder was converted

to methane via an aceticlastic pathway (Eq. 2.13). The Archaea 16S rRNA analyses demonstrated the hydrogenotrophic methanogens *Methanoculleus* spp. accounted for >90% of detected methanogens, and the acetotrophic methanogens *Methanosarcina* spp. were minor.

Aceticlastic cleavage

$$\underline{C}H_3COO^- + H_2O \rightarrow \underline{C}H_4 + HCO_3^- \quad \textbf{(Eq. 2.13)}$$

Non-aceticlastic cleavage

$$\underline{C}H_3COO^- + 4H_2O \rightarrow H\underline{C}O_3^- + HCO_3^- + 4H_2 + H^+$$

$$H\underline{C}O_3^- \text{ or } (HCO_3^-) + 4H_2 + H^+ \rightarrow \underline{C}H_4 \text{ or } (CH_4) + 3H_2O \quad \textbf{(Eq. 2.14)}$$

From their thermophilic (55℃) co-digestion of OFMSW and lipid-rich wastes, Martín-González et al. (2011) found *Methanobacterium*, *Methanoculleus* and *Methanosarcina* were detected. *Methanobacterium* and *Methanoculleu* belong to hydrogenotrophic methanogens, however *Methanosarcina* belongs to acetotrophic methanogens. No another acetotrophic methanogen, *Methanosaeta*, were detected in their result indicating *Methanosaeta* were unfavorable in thermophilic condition.

Tab. 2-2 Profiles of thermophilic methanogens (adapted from Suryawanshi et al., 2010)

No.	Methanogen	Site of occurrence	Cell morphology	Gram character	NaCl req. (M)	Substrate specificity	pH	Growth temp. (℃)	Reference
Genus: *Methanobacterium*									
1	*M. thermaggregance*	Mud from cattle pasture, Germany	Rod	－ve	NS	HC	6.5-9.0	40-75	Blotevogel and Fischer, 1985
Genus: *Methanocaldococcus*									
2	*M. jannaschii*	Submarine hydrothermal vent, East Pacific Rise, (2600 m depth)	Irregular cocci	NS	1.3-1.7	HC	5.2-7.0	50-85	Jones et al., 1983; Whitman, 2002a
3	*M. infernus*	Deep sea hydrothermal vent chimney, Mid-Atlantic Ridge (3000 m depth)	Cocci	NS	6.5	HC	5.2-7.0	55-91	Jeanthon et al., 1998; Whitman, 2002a
4	*M. fervens*	Deep sea hydrothermal vent core, Guaymas Basin, California	Regular and irregular cocci	NS	0.1-1.2	HC	5.5-7.6	48-92	Jeanthon et al., 1999; Whitman, 2002a
5	*M. vulcanius*	East Pacific Rise (2600 m depth)	Cocci	NS	1.5-14	HC	5.2-7.0	49-89	Jeanthon et al., 1999; Whitman, 2002a
6	*M. indicus*	Central Indian Ridge (2420 m depth)	Cocci	NS	0.75	HC	5.5-6.7	50-86	L'Haridon et al., 2003

Tab. 2-2 Continued on next page

Tab. 2-2 Continued 2

No.	Methanogen	Site of occurrence	Cell morphology	Gram character	NaCl req. (M)	Substrate specificity	pH	Growth temp. (℃)	Reference
Genus: *Methanoculleus*									
7	*M. thermophilus*	Sediment, Crystal River, Nuclear power plant, Florida	Irregular cocci	－ve	0.35-1.25	F, HC	6.1-7.8	55-65	Rivard and Smith, 1982; Maestrojuan et al., 1990; Spring et al., 2005
8	*M. receptaculi*	Shengli oil field, China	Cocci	NS	0.2	F, HC	7.5-7.8	50-55	Cheng et al., 2008
Genus: *Methanolinea*									
9	*M. tarda*	Municipal sewage sludge	Rod	NS	NS	F, HC	6.7-8.0	35-55	Imachi et al., 2008
Genus: *Methanomethylovorans*									
10	*M. thermophila*	UASB bioreactor, paper-mill wastewater, The Netherlands	Irregular cocci	－ve	0.1-0.3	Ma, Met	5.0-7.5	42-58	Jiang et al., 2005
Genus: *Methanopyrus*									
11	*M. kandleri*	Hydrothermal Rod heated deep sea sediment, California	Rod	＋ve	0.05-1.0	HC	5.5-7.0	84-110	Kurr et al., 1991
Genus: *Methanosaeta*									
12	*M. thermophila*	Thermal lake mud, Japan	Sheathed rod	－ve	NS	Ac	6.5-7.0	55-60	Patel and Sprott, 1990; Kamagata et al., 1992

Tab. 2-2 Continued on next page

Tab. 2-2 Continued 3

No.	Methanogen	Site of occurrence	Cell morphology	Gram character	NaCl req. (M)	Substrate specificity	pH	Growth temp. (℃)	Reference
Genus: *Methanosarcina*									
13	*M. thermophila*	Anaerobic digester (55℃), New York, USA	Cocci	NS	NS	Ac, HC, Ma, Met	6.0-7.0	50	Zinder et al., 1985
Genus: *Methanothermobacter*									
14	M. *thermau-totrophicus*	Anaerobic digester	Cylindrical irregularly rod	＋ve	NS	F, HC	5.0-8.0	45-70	Zeikus and Wolfe, 1972; Wasserfallen et al., 2000
15	*M. wolfeii*	Mixture of sewage sludge and river sediment, USA	Cylindrical irregularly rod	＋ve	NS	F, HC	6.0-8.2	37-74	Winter et al., 1985; Wasserfallen et al., 2000
16	*M. thermophilus*	NS	NS	NS	NS	NS	NS	NS	Laurinavichus et al., 1987; Boone, 2001
17	*M. defluvii*	NS	NS	NS	NS	NS	NS	NS	Kotelnikova et al., 1993; Boone, 2001
18	*M. thermoflexus*	NS	NS	NS	NS	NS	NS	NS	Kotelnikova et al., 1993; Boone, 2001
19	*M. marburgensis*	Mesophilic sewage sludge	Cylindrical irregularly rod	＋ve	NS	HC	5.0-8.0	45-70	Wasserfallen et al., 2000

Tab. 2-2 Continued on next page

Tab. 2-2 Continued 4

No.	Methanogen	Site of occurrence	Cell morphology	Gram character	NaCl req. (M)	Substrate specificity	pH	Growth temp. (℃)	Reference
Genus: *Methanothermococcus*									
20	*M. thermolitho-trophicus*	Biogas plant, Germany	Regular and irregular cocci	－ve	0.3-2.0	F, HC	6.5-7.5	30-70	Huber et al., 1982; Whitman, 2002b
21	*M. okinawensis*	Western Pacific deep sea, Okinawa Trough, Japan	Irregular cocci	NS	0.3-2.0	F, HC	4.5-8.5	40-75	Takai et al., 2002
Genus: *Methanothermus*									
22	*M. fervidus*	NS	Rod	＋ve	NS	HC	6.5	83	Stetter et al., 1981
23	*M. sociabilis*	NS	Rod	＋ve	NS	HC	6.5	88	Lauerer er al., 1986
Genus: *Methanotorris*									
24	*M. igneus*	Submarine vent, Kolbeinsey ridge, Iceland (106 m depth)	NS	NS	NS	NS	NS	45-91	Burggraf et al., 1990; Whitman, 2002c
25	*M. formicicus*	Black smoker chimney, Kairei field, Central Indian Ridge	Irregular cocci	NS	0.1-1.5	F, HC	6.5-8.5	53-83	Takai et al., 2004

＋ve: Positive; －ve: Negative; Ac: Acetate; F: Formate; HC: H_2 and CO_2; M: Molar; Ma: Methylamines; Met: Methanol; NS: Not specified

2.1.2 Factors and problems during operation: focusing on nutrients and sludge foaming

Nutrient balance is quite important in biotreatment, it has a significant influence on reactor operation if insufficient or improper ratios between macro- and micro-nutrients. Carbon, nitrogen, phosphorus, and potassium are common macro-nutrients, and demands of nutrients vary with different operation conditions. According to Khanal (2008), the theoretical minimum requirements that anaerobic system can be used are COD/N/P ratios of 350:7:1 for highly loaded (0.8-1.2 kg COD/kg VSS/d) and 1000:7:1 for lightly loaded (<0.5 kg COD/kg VSS/d). Many studies have also pointed out that some micro-nutrients like cobalt, copper, iron, molybdenum, nickel, selenium, tungsten, and zinc have considerable functions to anaerobic processes which associate with synthesis of enzymes, fatty acids metabolism, and conversion of CO_2/H_2 (McCarty, 1964b; Kayhanian and Rich, 1995; 賀延齡, 1998; Khanal, 2008). Certainly, previous experiments have further confirmed nutrients play a key role not only to methanogens but also to fermentative acidogenic bacteria (Kayhanian and Rich, 1995; Kim et al., 2003; Zitomer et al., 2008). Table 2-3 briefs some specific functions about macro- and micro-nutrients in anaerobic process.

Sludge foaming is a common problem not only in aerobic biotreatment but also in anaerobic biotreatment, and it will deteriorate the performances of treatment processes as well as increased the cost of operation. Ganidi et al. (2009) thought some reasons for causing AD foaming included surface active agents, filamentous microorganisms, temperature, organic overloading, type and frequency of mixing, and digester shape. The surfactants include oil, grease, volatile fatty acids, detergents, proteins and particulate matter. In these surfactants, proteins have a large influence to cause foaming because they are less biodegradable than lipids and fibers. In

Tab. 2-3 Functions of macro- and micro-nutrients in anaerobic digestion (Adapted from Kayhanian and Rich, 1995)

Macro-nutrients	Functions	Micro-nutrients	Functions
Carbon, C	Energy, cell material	Cobalt, Co	Corrinoids, CODH [a]
Nitrogen, N	Protein synthesis	Copper, Cu	SODM [b], hydrogenase
Phosphorus, P	Nucleic acid synthesis	Iron, Fe	CODH, precipitates sulfides
Potassium, K	Cell wall permeability	Molybdenum, Mo	FDH [c], inhibits SRB [d]
Sulfur, S	Numerous enzymes	Nickel, Ni	CODH, synthesis of F430, essential for SRB, aids CO_2/H_2 conversion
		Selenium, Se	Fatty acid metabolism, FDH
		Tungsten, W	FDH, may aid conversion CO_2/H_2 substrates
		Zinc, Zn	FDH, CODH, hydrogenase

[a] Carbon monoxide dehydrogenase
[b] Superoxide dismutase
[c] Formate dehydrogenase
[d] Sulfate reducing bacteria

general, sludge foaming caused by surfactants has two major factors that are interactions between compounds and between the compounds and solids in sludge could enhance or reduce the foaming potential, and the surface active agents are

broken down to simpler compounds during AD and are utilized by bacteria and therefore their impact on the foaming potential is unclear (Ganidi et al., 2009).

Gordonia spp. and *Microthrix parvicella* are considered that major causing foaming bacteria. These microorganisms are present in AD process via surplus activated sludge, they can exist in the liquid phase but also bound to the flocs. Filamentous microorganisms grow at the air/liquid interface of anaerobic reactors and produce biosurfactants, therefore, leading to lower surface tension of sludge and enhancing foaming possibility. Compared with mesophilic AD, thermophilic AD has more resistant to foam generation, this could be confirmed by the study of Han et al. (1997), which reported the extent of foaming was more moderate in TPAD system than in single-stage mesophilic anaerobic reactor. This suggested that high temperatures result in a lower surface tension and viscosity of sludge and hence increasing foam drainage (Ganidi et al., 2009).

2.1.3 General inhibitors of swine manure anaerobic digestion

In AD process, microbial inhibition may happen when the feed containing toxicants or, on the other hand, some specific by-products produced via metabolic processes. The common toxicants during anaerobic processes are ammonia, sulfides, salts, heavy metals and organics. Inhibition condition of the specific toxicant has significant differences in literature results due to inocula, waste composition, and experimental methods and conditions (Chen et al., 2008; Khanal, 2008).

2.1.3.1 Ammonia

In addition to feedstock containing ammonium, another nitrogenous source existing in anaerobic process is through degradation from proteins and urea of organic wastes, and ammonia inhibition will occur if its concentration exceeds the threshold

of microorganisms. From earlier studies we can find the toxic mechanisms of ammonia include a pH change of intracellular, increasing the maintenance energy requirement, and obstruction of a specific enzyme reaction (Chen et al., 2008). Ammonium (NH_4^+) and free ammonia (FA, NH_3) are the two major parts of inorganic ammonia nitrogen in aqueous solution, and their distribution is greatly affected by temperature and pH value expressed such as Eq. 2-15 and Eq. 2-16, which were according to Emerson et al. (1975); Østergaard (1985); and Koster (1986) (Hansen et al., 1997; Vandenburgh and Ellis, 2002).

$NH_3 = TNH_3 / [1+10^{(pKa-pH)}]$ **(Eq. 2.15)**

$pKa = 0.09018 + (2729.92/T)$ **(Eq. 2.16)**

Where

NH_3 = free ammonia concentration (mg/L as N);

TNH_3 = total ammonia concentration (mg/L as N);

Ka = equilibrium ionization constant; and

T = temperature (K).

Without a doubt, FA is regarded as the main reason causing inhibition since it may diffuse passively into the cell, causing proton imbalance, and/or potassium deficiency (Gallert et al., 1998; Chen et al., 2008). Methanogens have a poorer tolerance to ammonia inhibition than other anaerobic microorganisms, but this toxicity is reversible because the bacteria activity can be resumed immediately after high concentration of ammonia is diluted (賀延齡, 1998; Chen et al., 2008). Sensitivity results of ammonia was contradictory in previous studies, most investigations had found aceticlastic methanogens were more sensitive than

hydrogenotrophic methanogens on the basis of methane production and growth rate, however, a small portion of researches indicated aceticlastic methanogens had a relatively high resistance to high total ammonia nitrogen (TAN) level as compared to hydrogenotrophic methanogens (Chen et al., 2008). After arranging many literatures, Chen et al. (2008) thought there are several significant factors on ammonia inhibition, which were concentration, pH value, temperature, presence of other ions, and acclimation.

Angelidaki and Ahring (1993) investigated the influence of ammonia inhibition to thermophilic AD of cattle manure. They found inhibition occurred when the total ammonia concentration over 4 g N/L, but the reactor appeared steady-state after six months operation through TAN concentration of the reactor at 6 g N/L. From the results of specific methanogenic activity, it suggested the affect of ammonia toxicity to aceticlastic methanogens was deeper than hydrogenotrophic methanogens. Other reports confirmed that high temperature range strongly deteriorated the reactor performances due to presence of more unionized ammonia at high temperature, and reduction of temperature below 55℃ resulted in an increase of biogas yield and better process stability (Angelidaki and Ahring, 1994; Hansen et al., 1998).

Gallert and Winter (1997) evaluated mesophilic and thermophilic AD of household wastes and focused on effect of ammonia on glucose degradation and methane production. In their inhibition results indicated the thermophilic bacteria tolerated at least twice as much of FA than the mesophilic bacteria, in addition, the thermophilic was able to degrade more proteins. Another study from the same researchers, which investigated the effect of ammonia on protein degradation by mesophilic and thermophilic AD was again confirmed their view on ammonia toleration of thermophilic bacteria. Mesophilic AD revealed a higher rate of deamination than thermophilic AD when peptone concentrations from 5 to 20 g/L. If

0.5-6.5 g ammonia/L was added to the mesophilic AD, peptone degradation, chemical oxygen demand, as well as biogas production were inhibited, besides, no hydrogen was formed. Contrary to mesophilic AD, thermophilic AD was most active if existing approximately 1 g ammonia/L, and hydrogen was found in addition to methane (Gallert and Winter, 1998).

Sung and Liu (2003) found an improved methanogenic activity at lower TAN concentrations (<1.5 g/L), however higher TAN concentrations (>4.0 g/L) caused an obvious inhibition of methanogens. Although acclimation to high TAN levels had a poor methanogens activity, it still increased the tolerance of methanogens to ammonia and pH variations. A long-term study that investigated the interaction of temperature and ammonia in mesophilic anaerobic sequential batch reactors (ASBRs) for treating swine waste was implemented by Garcia and Amgenent (2009). Their results showed when the TAN were increased to approximately 4 g N/L, a 45% lower methane yield was observed at 25℃, and increasing the operating temperature from 25℃ to 35℃ improved the reactor performances. Furthermore, the acclimation ammonium concentration could exceed 5.2 g N/L for mesophilic anaerobic treatment of swine waste.

2.1.3.2 Sulfide and sulfate-reducing bacteria (SRB)

In addition to the industrial wastewater, swine wastewater is another common sulfur-containing waste due to existing large number of proteins. Sulfate-reducing bacteria (SRB), which can convert sulfur-containing wastes into sulfides in the anaerobic process have an abundant community not only in Archaea but also in Bacteria, they can be divided into four groups according to their types, physiological and biochemical characteristics, and 16 rDNA sequences: mesophilic Gram-negative SRB, thermophilic Gram-negative SRB, Gram-positive SRB, and sulfate-reducing

archaea, respectively (任南琪等, 2009).

SRB have two ways of inhibition in methanogenic process, these ways are Primary inhibition and secondary inhibition. Primary inhibition is the competition between SRB and methanogens because they use the same organic and inorganic substrates, on the other hand, secondary inhibition is attributed to the toxicity of sulfides produced via SRB metabolism. Different sulfides have varied toxic strength according to the molecular types is: H_2S > total sulfide > sulfite > thiosulfite > sulfate (Chen et al., 2008; Khanal, 2008; 任南琪等, 2009). Hydrogen sulfide has the highest toxicity because it can diffuse into the cell membrane, and it may have three toxicity mechanisms if H_2S penetrates into the cytoplasm. First, H_2S can change the protein structure by forming sulfide and disulfide cross-links between polypeptide chains. Second, it interferes with the various coenzyme sulfide linkages. And third, it also disturbs the assimilatory metabolism of sulfur (Chen et al., 2008). H_2S is strongly affected by the pH, H_2S concentration increases when pH<7, while pH ranging between 7 and 8, the concentration decreases obviously (McCarty, 1964c, d; 賀延齡, 1998). Certainly, corrosion and odor are also the problems concerning with hydrogen sulfide.

Researches confirmed both SRB and methanogens are utilization of acetate and hydrogen, thus this phenomenon would influence on the recovery of methane and the normal anaerobic process. Thermodynamics, kinetics, COD/SO_4^{2-} ratio, substrate types, pH, temperature, and adaption time are the crucial factors that can affect the competition between SRB and methanogens substantially (Khanal, 2008). A study about influence of ammonia and sulfate concentration on thermophilic AD was investigated (Siles et al., 2010). They found in terms of biogas, the threshold C/N and C/SO_4^{2-} ratios were 4.40 and 1.60, respectively, which correspond to 620 mg FA/L and 1400 mg SO_4^{2-}/L.

Fig. 2-5 is the relationship and substrate utilization between SRB and other anaerobic microorganisms. From the review paper of Chen et al. (2008), we can see four competitive relationships in the anaerobic process: competition between SRB and hydrolytic and fermentative bacteria; competition between SRB and acetogens; competition between SRB and hydrogenotrophic methanogens; competition between SRB and aceticlastic methanogens.

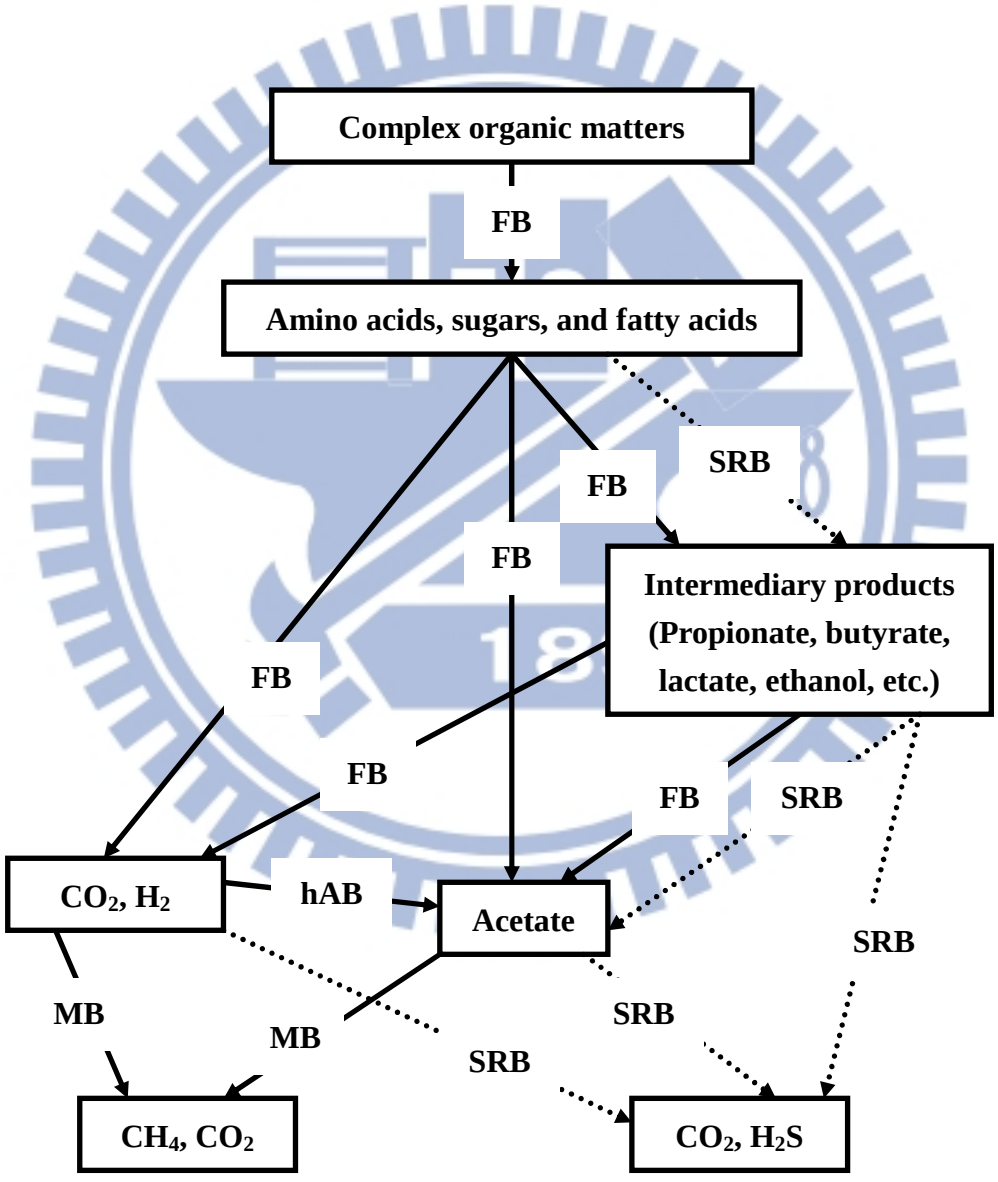


Fig. 2-5 The syntrophic relationship between SRB and other anaerobic microorganisms (Adapted from 任南琪等, 2009)
FB Fermentative bacteria; SRB Sulfate-reducing bacteria;
hAB Homoacetogenic bacteria; MB Methanogenic bacteria

2.1.3.3 Salts

Salts are important factors for growth of microorganisms. But when the concentration is higher than bacteria can't tolerate, and salts inhibition resulting from the dehydration of bacterial cells occurs due to osmotic pressure. Although salts are composed of cations and anions, the toxicity of salts was found to be predominantly determined by the cation (Chen et al., 2008). McCarty (1964c, d) found It occurred moderate inhibition when concentrations of Na^+, K^+, Ca^{2+} and Mg^{2+} are at 3500-5500, 2500-4500, 2500-4500 and 1000-1500 mg/L, respectively. 賀延齡 (1998) was on the basis of reports found the methanogenic activity reduced 50% at pH=7 and 35℃ when the individual salt concentration was Na^+=7600, K^+=6100, Ca^{2+}=4700 and Mg^{2+}=1930 mg/L, respectively. We can see the toxicity of divalent cations seems larger than monovalent cations. Chen et al. (2003) investigated the sodium inhibition of thermophilic methanogens. They found the specific methanogenic activity for aceticlastic methanogens acclimated to 0, 4.1, and 7.1 g Na^+/L ranged from 250 to 270 mg CH_4/g VSS/d, but the activity value was significantly decreased acclimated to 12.0 g Na^+/L, apparently, adaption to higher concentration of sodium could increase the tolerance of methanogens. Besides, in their chronic toxic result, the COD removal and methane production didn't appear significant deterioration when methanogens were acclimated to 12.0 g Na^+/L.

2.1.3.4 Heavy metals

High concentration of heavy metals also causes the inhibition to bacteria, and the ion-type is more toxic than. 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 in enzyme prosthetic groups (Chen et al., 2008). But if sufficient for sulfides in anaerobic

process, the heavy metals toxicity will reduce substantially due to forming non-solubility sulfide metal precipitates. Generally, 1.0 mg/L sulfides can be combined with 2.0 mg/L heavy metals to precipitate (McCarty, 1964c, d; Khanal, 2008).

2.2 The evolution from traditional AD to high-rate AD

The history of AD has been more than one century but the product of AD, methane, was found earlier by an Italian Volta in 1776, who knew this flammable gas would be generated via anaerobic decomposition of organic matter. The first full-scale applied anaerobic process was installed at France in 1860s. This facility was called “Muuras Automatic Scavenger” and it’s used to treat domestic wastewater, although its function was just like a septic tank. AD technique had a large advance should in the early 1900s because of a two-stage system known as Travis tank and Imhoff tank appeared, moreover, Imhoff tank was a modified type from former. A detached solids digestion made anaerobic treatment effectively to prevent the effluent from hydrolysis reactor and therefore the sludge would stay in the reactor from weeks to months until it became more stable.

Due to Imhoff tank was more economical on cost of sludge treatment, this facility was significant introduced, and from then on, AD technique would be shifted from treating wastewaters to treating solids. But AD technique didn’t become the main method of reducing pollution; contrarily, it faced with a limited situation before 1950 as people didn’t understand what happen in the AD process. Stander was the first man who realized the importance of solids retention time (SRT) for AD process in 1950. This concept promoted the development of high-rate anaerobic treatments which brought a separation of SRT and hydraulic retention time (HRT). Besides, it

made AD to apply in industrial wastewaters as well as biogas recovery. Short HRT could be achieved when SRT was still kept at long time and allowed the system operating at high organic loading rate without microorganisms' washout.

Many types of high-rate AD had been developed after in 1950, for instance, anaerobic contact process (ACP), anaerobic filter (AF), anaerobic membrane bioreactor (AnMBR), and upflow anaerobic sludge blanket reactor (UASB). AF and UASB processes established the immobilization of microorganisms and improved the mixing between sludges and wastewaters, follow-up reactors like anaerobic fluidized bed (AFB) and expanded granular sludge bed (EGSB) were the modified types basing on these characteristics. Other significant findings or inventions included that Speece recognized the importance of trace elements for methanogens in 1983; Dague and Pidaparti developed the anaerobic sequential batch reactor (ASBR) to treat swine manure in 1992. Tab. 2-4 is the evolution and main findings of the AD process. (賀延齡, 1998; McCarty, 2001; Khanal, 2008).

2.2.1 The development and application of TPAD

Due to USEPA formulated 40 CFR PART 503 regulations in early 1990s, the standard of biosolids as biofertilizers for cops has been stricter than the past. According to regulations, performances and operations of AD must reduce not only volatile solids (VS) but also pathogens, thus the digestate meets the Class A biosolids that VS removal should be more than 38%, fecal coliform should be less than 1000 MPN/g TS or *Salmonella* should be less than 3 MPN/4 g TS.

Many AD studies focus on this purpose to achieve reuse of digestion sludges, TPAD is also one of them and it was proposed by Dague and his co-workers at Iowa State University in 1993. The concept of TPAD was born from PhD thesis of Harris, who compared the performances of thermophilic (56℃) and mesophilic (35℃)

Tab. 2-4 Historical development of anaerobic biotechnology (Khanal, 2008)

Anaerobic technologies	Investigator(s) and place	Developments in chronological order
Discovery of methane	A. Volta, Italy	Recognized that anaerobic decomposition of organic matters produce methane (1776)
Mouras Automatic Scavenger	M. L. Mouras, France	Patented in 1881; the system had been installed in the 1860s
Anaerobic filter	Massachusetts Experimental Station, USA	Began operation in the 1880s
A hybrid system-a digester and an anaerobic filter	W. D. Scott Moncrieff, England	Constructed around 1890 or 1891
Septic tank	D. Cameron, Exeter, England	Designed in 1885 with provision for recovery of biogas for heating and lighting
	A. L. Talbot, USA	Designed in 1894 (Urbana); 1897 (Champaign)
Waste disposal tank	Leper colony, Matunga, Bombay, India	Digestion tank with gas collection system (1897)
Travis tank	W. O. Travis,	Development of a two-stage system for a separate solid digestion (1904)
Imhoff tank	K. Imhoff, Germany	Modified the Travis tank (1905)
Sludge heating system	Essen-Rellinghausen Plant, Germany	Development of first separate sludge digestion system (1927)
Sludge heating system	Essen-Rellinghausen Plant, Germany	Development of first separate sludge digestion system (1927)
Digester seeding and pH control	Fair and More	Realized the importance of seeding and pH control (1930)
High-rate anaerobic digestion	Morgan and Torpey	Developed digester mixing system (1950)
Clarigester (high-rate anaerobic processes)	G. J. Stander, South Africa	Realized the importance of SRT (1950)
Anaerobic contact process (ACP)	G. J. Schroepfer, USA	Developed ACP similar to aerobic-activated sludge process (1955)
Anaerobic filter (AF)	J. C. Young and P. L. McCarty , USA	Reexamined AF for the treatment of soluble wastewater (1969)

Tab. 2-4 Continued on next page

Tab. 2-4 Continued 2

Anaerobic technologies	Investigator(s) and place	Developments in chronological order
Upflow anaerobic sludge blanket reactor (UASB)	G. Lettinga, The Netherlands	Based on his first observation of granular sludge in Clarigester in South Afica (1979)
Expanded-bed reactor	M. S. Switzenbaum and W. J. Jewell, USA	Developed fixed-film expanded-bed reactor (1980)
Anaerobic baffled reactor	P. L. McCarty, USA	Retention of biomass within the baffles (1981)
Trace elements for methanogens	R. Speece, USA	Reported the importance of trace elements for methanogens activity (1983)
Anaerobic sequencing batch reactor (ASBR)	R. Dague and S. R. Pidaparti, USA	Developed ASBR for the treatment of swine manure (1992)

anaerobic biofilters to treat synthesis substrates and then Kiser and Dague reported a first study which combined thermophilic and mesophilic biofilters, the system COD removal could attain to 93% at 16 g COD/L/d and HRT=24 hr. This result achieved additional 48% COD removal than the mesophilic biofilter in Harris' study, even though both reactors operated at the similar loading rate. Following the Kaiser's research, the temperature-phased method integrated with ASBR technique that had been investigated by Dague and his co-workers many years and gained well performances at either high or low waste concentrations; subsequently, TPAD studies were major in producing Class A biosolids by Sung and his group (Welper et al., 1997; Han et al., 1997; Sung and Santha, 2003; Santha et al., 2006).

As Fig. 2-6 shown in, TPAD is one of two-stage AD systems combining thermophilic AD and mesophilic AD, taking thermophilic-phased advantages like high solids removal, more biogas production and effective pathogens elimination as well as mesophilic-phased advantages improving system's stabilities, reducing odorous problems and polishing digestates in one system offsets the drawbacks appeared when thermophilic or mesophilic reactors are operated individually.

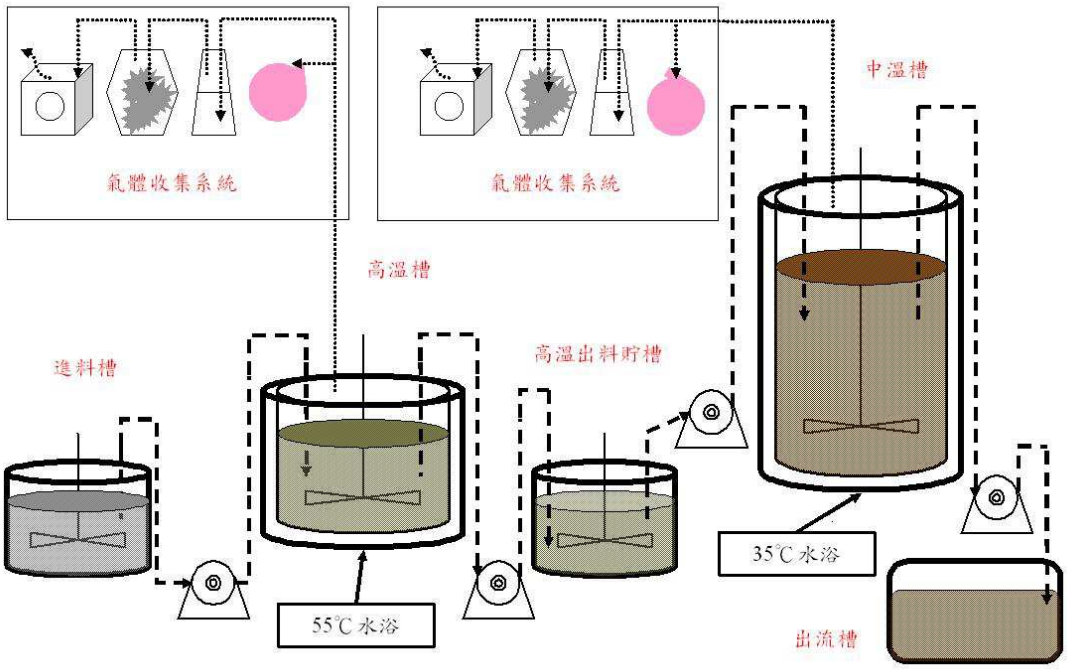


Fig. 2-6 Schematic diagram of temperature-phased anaerobic digestion (TPAD) system

TPAD has significant capacities in sludge digestion; in addition, it provides a safe sanitation once pathogens are eliminated. Current researches about TPAD have been growing obviously, and mostly investigate to treat sewage sludge. According to master's thesis of Li (2004), there have been more than 15 full-scale facilities applied in wastewater treatment plants (WWTP). Sewage sludge, particularly waste activated sludge (WAS) which is residual through the aerobic biological treatment is very difficult further degraded and needs extra adjustments before it enters the next step which is usually traditional single mesophilic AD, thus the cost for treating sludge increases apparently. However many studies confirm TPAD has a lot benefits in digesting sewage sludge even there isn't any adjustments in the sludge. WWTP sludge digestion isn't the only one benefited by TPAD, its performances of treating others containing high solid strength like food processing industry wastes, agriculture wastes, livestock wastes, and slaughtering industry wastes may be surprising.

Because high temperature results the low pathogenic risk digestate, this product can use as biofertilizers or soil conditioners to bring a feedback on farming. Consequently, we have to deeply recognize emphases of TPAD in particular thermophilic anaerobic process due to its existing defects lead to a restrictive application.

Compared with mesophilic AD, the application of thermophilic AD is not widely. The main reasons have four possibilities. First, thermophilic methanogens grow slowly so need more time in operation or the reactor can't achieve steady state. The start-up time of mesophilic AD takes two to four months in general, however thermophilic AD will take more time approximately six months to a year to maintain reactor stability because thermophilic methanogens have a high decay rate and these bacteria are fewer in mesophilic inoculum sludges. Second, microbes has a tendency to accelerate their metabolism at high temperature situation, thus producing numerous intermediates like volatile fatty acids (VFAs), this also causes high VFAs and COD concentrations in many thermophilic reactors instead of in mesophilic reactors. Third, some toxicants concentrations, especially ammonia, increase apparently in high temperature range due to ammonia transforms into undissociated type known as free ammonia (FA); FA concentration is associated with temperature and pH. Finally, it should add more energy on heating to maintain high temperature, thus thermophilic AD often applies what influent temperature is high and it rarely uses in high-latitude areas. On the other hand, some defects may regard as advantages. Thermophilic methanogens have a high decay rate but it also indicates the sludge production in the thermophilic reactor is much less than in the mesophilic reactor. In addition, more biogas production is not only enough holding the reactor temperature but providing for the heat and power generation (CHP) system. Moreover, high temperature eliminates most pathogens and this will improve the digested sludge availability.

The concept of two-stage anaerobic step may accompany with the appearance of Imhoff tank, and it has a great relationship with anaerobic microbial metabolic pathways. Hydrolytic fermentative bacteria decompose complex organic matters producing acetate, carbon dioxide and hydrogen, and then these intermediates are synthesized to methane by methanogens. So we distribute certainly the two-stage AD to an acidogenic reactor and a methanogenic reactor due to differences of growth characteristics between acidogenic bacteria and methanogens. TPAD is one of two-stage AD but separating two parts depends on the reactor temperature, but TPAD can be categorized as either acidic thermophilic TPAD (AT-TPAD) or neutral thermophilic TPAD (NT-TPAD) by adjusting pH of thermophilic reactors. Lv et al. (2010) compared performances of AT-TPAD and NT-TPAD from previous studies, they found NT-TPAD had better performances when treating the same substrates (PS and OFMSW) at the similar organic loading rate and HRT, whereas other researches reported AT-TPAD had well effects in cellulose hydrolysis. It seems that AT-TPAD and NT-TPAD still have quite contradictory results in their performances, more investigations need about these AD systems in the future. Tab. 2-5 showed comparisons of reactor performance and operation between NT-TPAD and AT-TPAD.

Tab. 2-5 Comparisons of VS removal and reactor operation between NT-TPAD and AT-TPAD

System type	Reactor operation (T/M, ℃)	Substrate type	VS conc. (%)	HRT (d) T/M/system	OLR (g VS/L/d) T/M/system	VS removal (%)	Reference
NT-TPAD	SC (55/35)	PS + WAS	4.0% (TS)	4/10/14	7.3/2.1/—	45%	Han et al., 1997
	ASBR (55/35)	Dairy WW	—	0.6/2.4/3	20/—/4.0	44%	Dugba and Zhang, 1999
	ASBR (55/35)	PS + WAS	4.9%	7.4/12.6/20	5.1/—/1.9	62%	Vandenburgh and Ellis, 2002
	SC (55/35)	Dairy cattle waste	8.0%	4/10/14	—/—/5.8	42%	Sung and Santha, 2003
	ASBR (56/36)	PS + WAS	5.0%	8/8/16	—/—/2.7	53%	Santha et al., 2006
	SC (55/35)	PS + WAS	2.4%	3/12/15	7.8/1.5/1.6	85%	Riau et al, 2010b
	ASBR (55/35)	Sewage sludge + food waste	4.2%	—/—/7	—/—/6.1	45%	Kim et al., 2011
AT-TPAD	SC (55-60/37)	PS + WAS	—	2/10/12	14.5/2.1/2.4	61%	Huyard et al. 2000 [a]
	CSTR 55/35	PS + WAS	3.0%	3/10/13	11.3/3.0/4.8	32%	Rubio-Loza and Noyola, 2010
	SC 55/35	Microwaved sludge	3.9%	2/8/10	26.2/3.4/3.9	50%	Coelho et al., 2011

[a] pilot-scale

2.2.2 Start-up and operation of TPAD

TPAD system combines thermophilic and mesophilic AD, however the stability of this system depends on the effect of thermophilic reactor. The main problem which results in a large trouble for TPAD system set-up is taking much time to accumulate methanogens of a thermophilic reactor as well as a mesophilic reactor. Methanogens grow slowly than acidogenic bacteria. If organic loading rate exceeds the system threshold, VFAs concentration will increase significantly and decline the pH. As a result, methanogens are inhibited by the acidic pH and toxic VFAs. To avoid the drop of pH, we usually supply extra alkaline materials, for example $NaHCO_3$, keeping the pH in a safe range, this method is quite common if we use rich in carbohydrate as the substrate. Besides we should pay attention to alkalinity, the inoculum sludge concentration is another key point we must concern because it determines the total operation time.

It is ideal and shortens the operation stage significantly if we can directly obtain the thermophilic inoculum sludge from a full-scale thermophilic AD plant. But if we can't get the sludge, another choice that accumulate mesophilic inoculum sludge in a high temperature condition can be accepted. 賀延齡 (1998) pointed out the range of optimal growth temperature is the inherent characteristics of bacteria themselves. Only 9% thermophiles and 1% obligate thermophiles were present and appeared dormancy in mesophilic sludge from previous study (賀延齡, 1998; Boušková et al., 2005). As a result using mesophilic sludge as inoculum for thermophilic methanogens exist a large challenge due to thermophilic bacteria are rare, and there have two strategies for increasing them which are step-wise method and one-step method, respectively. Although one-step method appears serious interferences at initial start-up period, reactors will reach a new steady state after about 30 days operation. Moreover, one-step method can save half of the

accumulation time, this method is recommended an optimal accumulation strategy for thermophilic bacteria using mesophilic sludge. If we take step-wise method to strengthen thermophilic bacteria, the temperature change of every phase should not exceed 5℃ (Bouškovᡠet al., 2005).

2.2.3 Performance of volatile solids removal

As most traditional AD treatment, TPAD has been widely applied to sewage sludge or OFMSW (Han et al., 1997; Oles et al., 1997; Roberts et al., 1999a, b; Huyard et al., 2000; Vandenburgh and Ellis, 2002; Song et al., 2004; Santha et al., 2006; Riau et al., 2010a, b; Rubio-Koza and Noyola, 2010; Ge et al., 2010; Ge et al., 2011; Coelho et al., 2011), and secondly applied to livestock wastes (Dugba and Zhang, 1999; Sung and Santha, 2003), and also some applied to co-digestion of sewage sludge and other wastes (Lafitte-Trouqué and Forster, 2000; Kim et al., 2011). Organic wastes reduction is the main function of AD, and evaluating this effect is through VS removal instead of COD removal if using high solid concentration wastes. The digestate if using as biofertilizer should meet the standard of Class A biosolids, which is no pathogen risks and VS reduction achieves above 38% (U.S. E.P.A., 1993).

TPAD systems treating sewage sludge has been confirmed they have excellent effects by previous studies. For example, Han et al. (1997) found compared with the single mesophilic control, TPAD systems just needed 14-day HRT to attain 38% VS removal yet the former at least needed the double HRT to reduce VS. The shorter HRT using in TPAD system indicates the reactor volume of TPAD can be smaller than a single mesophilic reactor when treating the same substrate at a given OLR. Oles et al. (1997) investigated the full-scale two-stage AD treatments, which were also known as the TPAD system in Germany. They found the full-scale

thermophilic/mesophilic digestion process had significant improvements not only in VS removal but also in biogas production when they changed the process from traditional operation to temperature-phased operation.

The TPAD performance of varying solids concentration and OLR was investigated (Vandenburgh and Ellis, 2002). OLR can be adjusted by changing HRT or feeding concentration, researchers changed the solids concentration and fixed the HRT to increase the system OLR. They found the VS removal of TPAD system was above 60% when solids concentration was under 4.9% VS, besides, 50% VS removal could achieve even though the VS concentration was high as 7.9%. They concluded the performance of TPAD VS removal greatly depended on the thermophilic reactor and VS removal of mesophilic reactor didn't affect significantly because polishing was the main function at this stage. VS removal was contradictory to studies of Riau et al. (2010a, b). In their discontinuous study, the effect of thermophilic VS removal was similar to the study of Vandenburgh and Ellis (2002): most solids were reduced in the thermophilic stage. But in their semi-continuous study, system VS removal depended on the performance of mesophilic stage, it might be the thermophilic reactor appeared VFAs accumulation and influenced the VS reduction in this stage.

TPAD system combines with sequential-batch reactors was investigated, and had positive results at municipal sludge as well as co-digestion with other wastes. SBR operation can separate SRT and HRT, moreover, shorten the HRT to increase the reactor OLR and simultaneously maintain the sludge at a longer SRT. Dugba and Zhang (1999) applied TPAD and SBR system to treat dairy wastewater, they found the two-stage SBR system were suitable for treating this waste with short HRT, and recommended to keep the OLR between 2 and 4 g VS/L/d if the system was operated at the 3-day HRT. Santha et al. (2006) investigated the performance of

TPAD and SBR system treating municipal sludge, they demonstrated this process was more stable than a conventional single stage and didn't show any effects of shock loading during operation. Another study about TPAD and SBR system was accomplished by Kim et al. (2011), they used co-digestion of sewage sludge and food waste as the feed. Co-digestion which mixes two or more different source wastes to achieve nutrient balance also improves the stability of AD process, and is a research priority of AD process recently. Researchers found the system allowed a higher VS removal indicating this system had a better balance conversion from organics to CH_4 at high OLR of about 6.1 g VS/L/d.

To increase solids hydrolysis of TPAD system, some researchers allow pH of thermophilic reactor is acidic, this system is also called AT-TPAD. Thermophilic reactor of AT-TPAD is sometimes called acidogenic reactor which producing organic acids is the main function, and mesophilic reactor of AT-TPAD is called methanogenic reactor which transforms organic acids and intermediates into methane. Performances of AT-TPAD was also evaluated by many studies (Huyard et al., 2000; Rubio-Koza and Noyola, 2010; Coelho et al., 2011). These studies had moderate effects and were full of contradictions when it compared with NT-TPAD. Other improvements of TPAD contained reactors modification (Roberts et al., 1999b; Song et al., 2004), increasing temperature in the thermophilic stage (Roberts et al., 1999a; Ge et al., 2010; Ge et al., 2011), and adjusting wastes (Lafitte-Trouqué and Forster, 2000; Coelho et al., 2011; Kim et al., 2011).

2.2.4 Performance of production and component of biogas

The biogas production of TPAD system was confirmed it had a large potentiality due to high VS reduction, and both NT-TPAD and AT-TPAD systems have a large number of biogas comparing with traditional single-stage reactors.

NT-TPAD produces more biogas in the thermophilic reactor than in mesophilic because the former is the main performance of this two-stage system. However the responsibility for producing biogas in AT-TPAD system is mainly by the post-treatment reactor, mesophilic methanogenic reactor, because the thermophilic reactor is responsible for generating the precursors of methane.

In treating sewage sludge, many studies indicated that high temperature first-stage improved the decomposition of sewage sludge, particularly waste activated sludge (WAS), thus resulting in more biogas production. Han et al. (1997) pointed out that TPAD system achieved a methane production rate approximately 30 to100% higher than single-stage mesophilic reactor. A full-scale TPAD system in Germany also supported this result, researchers found temperature-phased operation increased 16.5% biogas production than past mesophilic operation although the gas yield was still low due to few organic fraction in the raw sludge (Oles et al., 1997).

Vandenburgh and Ellis (2002) found both thermophilic and mesophilic biogas production increased with feed sludge concentration, and interestingly, when TS concentration exceeded 4.9%, the biogas production of mesophilic reactor was higher than thermophilic reactor. From their VFAs data, VFAs concentration was below 1000 mg/L when sludge concentration was below 4.9%, therefore, thermophilic reactor could consume these organic acids and no VFAs accumulation problem. But thermophilic reactor couldn't consume VFAs immediately at high sludge concentration. As a result, follow-up mesophilic reactor was responsible for degrading VFAs producing from thermophilic reactor and then led to much biogas in mesophilic reactor. This procedure made NT-TPAD system similar to AT-TPAD system since the high OLR resulted in VFAs accumulation as well as a drop pH. The biogas performance of AT-TPAD system is still high than single-stage mesophilic AD, however compares with NT-TPAD system, its overall performance

may be unsatisfying and make system at unstable status (Rubio-Koza and Noyola, 2010; Coelho et al., 2011). Even if VFAs accumulation appears at high OLR, the HRT of thermophilic reactor doesn't be recommended due to reducing system efficiency. Riau et al. (2010b) could verify this view from their study, it suggested that the efficiency of the thermophilic reactor was lower than the mesophilic reactor if operated at the same long HRT.

In treating cattle waste, Dugba and Zhang (1999) found the methane production at first thermophilic stage of all systems was higher than second mesophilic stage, indicating mesophilic stage could be operated at high OLR or we could reduce the volume of the mesophilic reactor. Sung and Santha (2003) increased solids concentration to adjust system OLR. They found methane production rates from thermophilic stage were higher than the mesophilic reactor due to high VS removal in the thermophilic stage. Compared with thermophiloic reactor, the methane yield of mesophilic reactor was larger at all OLR, suggesting the thermophilic reactor didn't converted intermediates to methane effectively.

Methane and carbon dioxide are the main composition of biogas in AD process, and with other small amount of gas like nitrogen and hydrogen sulfide. According to previous studies, the difference of biogas composition wouldn't be significant whether researchers used thermophilic AD or mesophilic AD treatment, however, methane content of thermophilic AD was a little less than mesophilic AD. The most important factor that affects methane content is composition of the substrate.

The biogas composition of treating sewage sludge by TPAD system was that the thermophilic reactor had a methane content of 41-68%, carbon dioxide of 27-30%, nitrogen of 3-5%, and hydrogen sulfide of 150 ppm; the mesophilic reactor had a methane content of 53-72%, carbon dioxide of 24-27%, nitrogen of 2-5%, and hydrogen sulfide of 25 ppm (Han et al., 1997; Li, 2004; Song et al., 2004; Santha et

al., 2006; Riau et al., 2010b; Rubio-Loza and Noyola, 2010; Coelho et al., 2011). And the biogas composition of treating cattle manure at varying OLR by TPAD system was that the thermophilic reactor had methane content of 58-61%, and hydrogen sulfide of 500-1300 ppm; the mesophilic reactor had a methane content of 59-62%, and hydrogen sulfide of 125-700 ppm. Like other studies, carbon dioxide was the second only to methane (Sung and Santha, 2003). It seemed that treating sewage sludge and cattle manure had similar results, but noting the hydrogen sulfide concentration in digesting cattle manure was higher than in digesting sewage sludge suggesting livestock wastes have a large number of proteins, and lead to a higher hydrogen sulfide concentration.

Besides substrates are the major influence of biogas composition, the reactor operation is also critical. For instance, a modified TPAD system treating co-digestion of sewage sludge and confectionery waste was investigated, researchers found the average methane content of mesophilic methanogenic reactor was about 44-82% with mesophilic HRT decreasing from 15-day to 8-day, the thermophilic reactor was a pre-treatment stage which HRT was fixed at 4-hour (Lafitte-Trouqué and Forster, 2000). Furthermore, Şentürk et al. (2010) studied the performance of treating potato-chips wastewater by thermophilic anaerobic contact reactor, and they found the methane content declined gradually from 89% to 68%, while the reactor OLR rose from 0.6 to 8.0 kg COD/m^3/d.

2.2.5 Performance of pathogens removal

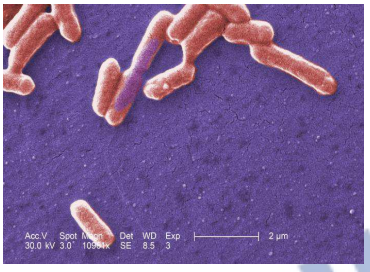
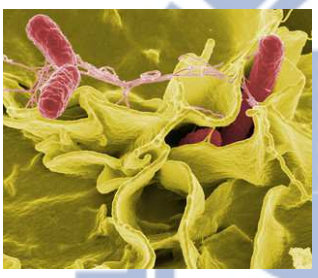
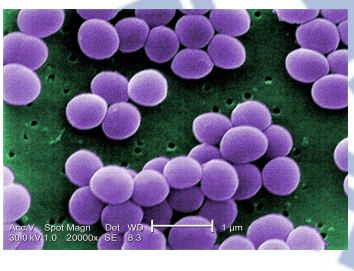
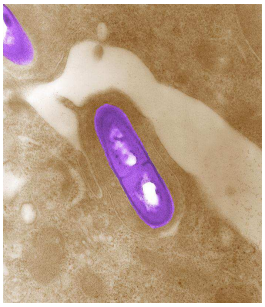
Pathogens elimination is a crucial factor whether the digested sludge can use in agriculture. For disinfection standards of anaerobic biological process, temperature and residence time of reactors are the main impact operation parameters. Disinfection can be explained that exposure time at 70℃ is at least 30-min or at

55℃ is above 4-hour. As a result, for the temperature range of thermophilic AD is mostly at 50-60℃, the minimum of HRT for 55℃ is at 4-hour or for 60℃ is at 3-hour (Roberts et al., 1999a, b). Certainly, high temperature is an emphasis on disinfection, high VFAs concentration and low pH range are significant for pathogens inactivation (Salsali et al., 2006; Riau et al., 2010b). In addition, if substrates are rich in proteins, these large amounts of organic nitrogen will transform into ammonium via ammonification. Ammonium can also exist with un-ionized type at high temperature and pH which is more toxic for microorganisms, therefore, the un-ionized ammonium concentration in thermophilic AD is higher than in mesophilic AD (Vandenburgh and Ellis, 2002).

For reduction of pathogens through TPAD system, most studies have confirmed it will have effective results under the optimal ranges of OLR and residence time (Han et al., 1997; Dugba and Zhang, 1999; Huyard et al., 2000; Song et al., 2004; Riau et al., 2010a, b; Coelho et al., 2011). The pathogens removal standards of Class A biosolids must meet fecal coliform should be less than 1000 MPN/g TS or *Salmonella* should be less than 3 MPN/4 g TS. But fecal coliform and *Salmonella* are not the only pathogens we concern, others like *Staphylococcus aureus*, *Listeria monocytogenes*, and *Campylobacter* spp. may also be potentially dangerous and appear pandemic easily (FiBL, 2011). Tab. 2-6 is about some features and influences on human health of these pathogens.

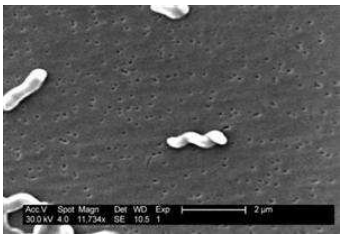
Aitken et al. (2007) evaluated the inactivation of the pathogenic *Escherichia coli* O157:H7 and a non- pathogenic *E. coli* strain isolated from thermophilic AD of cattle manure at 50 and 55℃ batch tests. They found inactivation rates of heat-sensitive fractions was similar for both colony types at each temperature, indicating *E. coli* could be used as an indicator of inactivation of serotype O157:H7. However, it could lead to misinterpretation of inactivation kinetics and could result

Tab. 2-6 Five typical types of common pathogens in manure and agriculture wastes (Adapted from Koller, 2011; figures from: Centers for Disease Control and Prevention, C.D.C and National Institutes of Health)

Pathogens	Remarks
Escherichia coli 	*E. coli* are rod-shaped, Gram-negative bacteria, which are natural and generally harmless inhabitant of the lower intestine of humans and animals. Thus, they are an important indicator of fecal contamination. Some strains can cause serious and even life-threatening complicantions such as hemolytic-uremic-syndrome (HUS).
Salmonella serovars 	*S. serovars* are rod-shaped, Gram-negative, non-spore forming bacteria and are the bacterial foodborne pathogens most commonly linked to outbreaks. Infection leads to diarrhea that can be life-threatening to labile persons and children. This bacterium can survive outside the body of its host for weeks and was found in dry fecal matter for over 2 years, poultry and eggs are often contained with *Salmonella*.
Staphylococcus aureus 	*S. aureus* are ball-shaped, Gram-positive bacteria and they often appear in clusters. They are ubiquitous in nature and certain strains can cause a wide-range of diseases, from minor skin infections to sepsis. They can produce enterotoxins, which are heat stable and not destroyed by cooking, causing diarrhea and vomiting.
Listeria monocytogenes 	*L. monocytogenes* are rod-shaped, Gram-positive, motile bacteria. They can be found in soil, water, plants and animals and are classical foodborne pathogens. They can cause serious infections in newborns, pregnant and immunocompromised persons. The symptoms range from diarrhea to live threatening meningitis and encephalitis in labile people.

Tab. 2-4 Continued on next page

Tab. 2-4 Continued 2

Pathogens	Remarks
Campylobacter spp. 	*Campylobacter* spp. are spiral-shaped, Gram-negative bacteria that are sensitive to oxygen and dry conditions. They can be found in many animals. Some strains are poorly suited for growth in food, but the number of bacteria required for food poisoning on the other hand is low.

in incorrect decision if using plating methods with differential-selective agars to calculate *E. coli* concentration, because low concentration non-target organisms can grow on the media.

The detection of pathogens has been limited due to different culture media used with different strains, and it wastes much more time and energy to detect these microbes. Therefore, fecal coliform, coliform group and *Salmonella* are commonly used as pathogen indicators, and they also exist in livestock wastes. However, there are still have many reports concerning with other pathogens removal results like *Listeria* (Burtscher et al., 1998; Nicholson et al., 2005), *Compylobacter* (Nicholson et al., 2005), *poliovirus* (Huyard et al., 2000), and helminth eggs (Huyard et al., 2000; Rubio-Loza and Noyola, 2010) were studied. Burtscher et al. (1998) found *Listeria* had a significant removal as well as their *Salmonella* result. Huyard et al. (2000) found the destruction of fecal coliform, *poliovirus*, and helminth eggs were 5.5 log, 4.0 log, and 2.6 log, respectively, besides, they thought the destruction of helminth eggs were relevant to inactivation and lysis of the egg. Rubio-Loza and Noyola (2010) used the same AT-TPAD process like Huyard et al. (2000), and had similar pathogen destructions in fecal coliform and helminth eggs.

A paper which compared pathogens survival in livestock manure during storage and following land application was executed through field experiments (Nicholson

et al., 2005). They found *E. coli O157*, *Salmonella*, and *Campylobacter* survived in stored slurries and dirty water for up to three months, and *Listeria* could survive up to six months for a long time. In contrast, pathogens could only survive for less than a month in solid manure heaps because composting process occurs where temperature is higher than 55℃. When following manure spread to land, *E. coli O157*, *Salmonella*, and *Campylobacter* survived for approximately one month in the soil, *Listeria* could survive for more than one month due to commonly found in soil.

2.3 Co-digestion and sustainable utilization of livestock waste

Anaerobic co-digestion is one emphasis that treats high solid concentration wastes in the recently, according to Mata-Alvarez et al. (2011), papers having the title about co-digestion have been gradually increased since 1995. Co-digestion is defined after homogenizing and adjusting two or more varying sources of organic wastes and then takes this slurry as a feedstock of anaerobic treatment. Occasionally, AD treating single source waste has a poor performance and causes system unstable due to nutrient imbalance, containing toxicants or producing a large amount of inhibitors via microbial metabolism. Apart from the types of organic wastes, the C/N ratio is another significant influence on performances of AD, if carbohydrate-rich waste comes into the anaerobic process, the performance will decline due to producing more VFAs which result in a rapid alkalinity consumption and then dramatic pH drop; however when the waste containing considerable nitrogen, like proteins and urea, will make a poor efficiency because of existing high concentration ammonium. Fig. 2-7 is a distribution of papers dealing with co-digestion of varying wastes according to their quantity and C/N ratio. We can

find the theme of most literatures have focused on sewage sludge and manures, and the second were OFMSW and industrial wastes, meat industries and animal wastes, agricultural wastes as well as crops were the research topics that fewer people concerned (Mata-Alvarez et al., 2011).

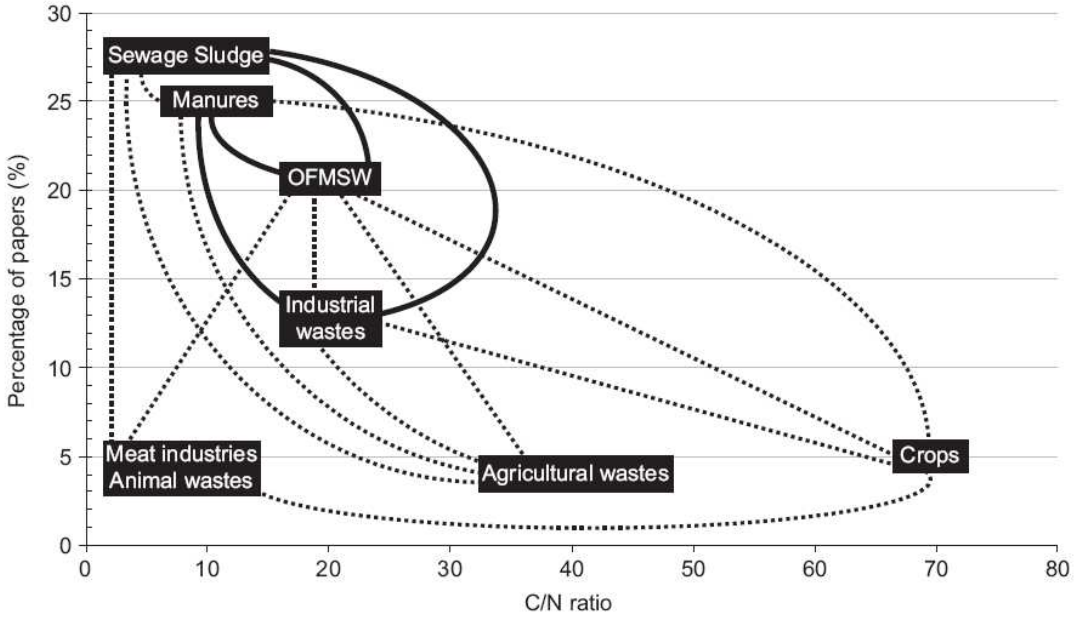


Fig. 2-7 Source distribution of co-digestion wastes in literatures (Mata-Alvarez et al., 2011)
Solid line: the most reported mixtures; dotted line: other published co-digestions

2.3.1 Anaerobic co-digestion: case studies with different substrates

Kaparaju and Rintala (2005) evaluated the co-digestion feasibility using the potato tuber and its industrial by-products with swine manure. Reactor type was continuous digestion stirred tank (CSTR) and operation conditions were at 35℃, HRT=20 days and loading rate of 2.5 kg VS/m^3/d. The methane yields were 0.13-0.15 at 100:0 (VS% pig manure to VS% potato co-substrate), 0.21-0.24 at 85:15 and 0.30-0.33 at 80:20 feed ratios. Their results showed a successful operation could be achieved with co-digestion pig manure and potato waste and

provided an opportunity digesting livestock manure with other similar industrial residues.

Lansing et al. (2010) used a low-cost digester, plug-flow reactors (250 L each) operating without mechanical or heating installations to assess reactor performances and values of fertilizer, which were co-digestion of swine manure and used cooking grease. Four tests were carried out: the control (T0), which only contained swine manure, and T2.5, T5, and T10, which contained 2.5%, 5%, and 10% used cooking grease (by volume) combined with swine manure. Furthermore, the local temperature were approximately 22-26℃ during the nine-month experiment period (May 2007-Februbay 2008). Rresearchers found T2.5 had the greatest methane production (45 L/d), a 124% increase from the control, and without any deterioration was observed in terms of VS removal, pathogens reduction, grease removal as well as pH. Total nitrogen concentration decreased 34.0%, and on the other hand NH_4^+-N increased 97.1% during T2.5, with no significant differences between T2.5 and T0. However, compared with T0, co-digestion runs had a less phosphorus reduction. The total phosphorus concentration was 181 mg/g in T2.5 and only 90.6 mg/g in T0.

A study of thermophilic anaerobic co-digestion was achieved by Cavinato et al. (2010), who used cattle manure, agro-wastes and energy crops as a co-substrate. From the results, they suggested a proper thermophilic condition (55℃) had improvements not only in biogas production but in stability of digestion process. In addition, from economic aspect, the net present value of the investment, considering only the AD, was 2.5 years. If we also considered the treatment for nitrogen removal, the net present value of the investment was 3-5 years depending on the efficiency of nitrogen removal.

According to the study of Zhang et al. (2011), using food waste and piggery

wastewater as a co-substrate, once again confirmed nutrient and trace element balance which is an advantage of co-digestion significantly improved biogas production and stabilized AD process. Besides, the analytical results indicated Korean food waste contained higher energy potential and lower concentration of trace elements than the piggery wastewater.

2.3.2 The concept and development of Biogas plants

Biogas plant is an important concept which achieves bioenergy production, organic waste management, and nutrient recycling and redistribution by anaerobic process, in general there are two categories of biogas plant known as joint biogas plant and farm scale biogas plant (Raven and Gregersen, 2007; Holm-Nielsen et al., 2009). Joint biogas plants, also called centralized plants, co-digest animal manure collected from several farms, mixed with other organic waste sources like agriculture and food wastes, and they are usually of large scale, with digester capacities ranging from few hundreds m^3 to several thousands m^3, Fig. 2-8 is the main streams of centralized co-digestion plant. As its name implies, farm scale biogas plants co-digest animal manure and other organic wastes from one single farm or, rarely two or three smaller neighboring farms (Holm-Nielsen et al., 2009).

EU-countries has a leading position in the development of biogas plants, and where the agricultural biogas plants are most developed are Germany, Denmark, Austria and Sweden and to a certain level the Netherlands, France, Spain, Italy, United Kingdom and Belgium. Portugal, Greece and Ireland as well as in many Eastern European countries have a large development possibility because their large amount agriculture and livestock wastes (Holm-Nielsen et al., 2009).

A case study about Biogas plants in Denmark was investigated by Raven and Gregersen (2007). They assessed 20 centralized and over 35 farm-scale plants and

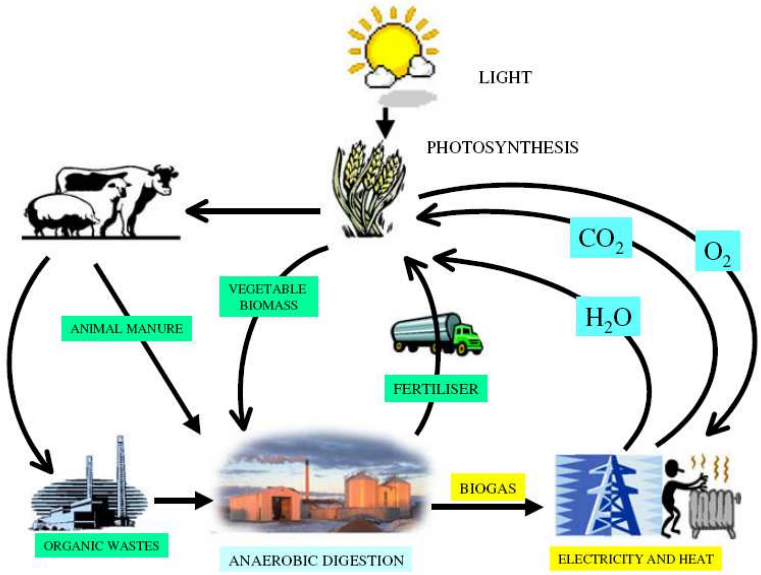


Fig. 2-8 Sustainability of anaerobic co-digestion (Holm-Nielsen et al., 2009)

found the co-digestion of manure and organic waste had a well established technological practice in Denmark, however, the development of these plants didn't appear without difficulties. They thought the setback in biogas plants was mainly caused by a shift in energy and environmental policies and limited availability of organic waste.

Chapter 3 Materials and methods

3.1 Experimental design start-up of TPAD, and reactor operation

Fig. 3-1 is the flowchart of TPAD co-digestion with pig manure (PM) and rice straw (RS). This study focused on VS concentration and the ratios of PM and RS that are the two crucial factors on reactor operation. However, due to the limitation of laboratory equipment, higher VS concentration runs couldn't carry out with this TPAD system. To avoid the feed pump appearing serious obstruction, the maximum VS concentration didn't exceed 20 g VS/L, the pump obstruction still occurred in the whole operation period even under this VS range. Also to avoid the obstruction caused by RS, this study just carried out two run, which the RS percentage contributing to VS concentration were only 20% or 10%. The mixing ratio was depended on the contribution of individual VS concentration of PM and RS to the thermophilic goal feed concentration.

The TPAD system using PM and RS as the co-digestion substrate was operated 585 days and had some preliminary results at reactor performances and the feasibility using the final effluent as a bio-fertilizer. The total operation period took about 474 days to achieve the goal HRT (T/M=4/10 d). The biggest reason that took more than a year to domesticate thermophilic anaerobes was the obstruction resulted from unsuitable pumps when pumping the feed containing high solids concentration. And thereby affecting the accumulation of thermophilic anaerobes, not to mention, these thermophilic microorganisms were very difficult on the domestication even without the problems of equipment.

585 days operation was adjusted in accordance with reducing HRT, increasing

feed concentration, and changing ratios of PM and RS, here was a description about change of the operating parameters as follows. Operation in 0-125 days was the first operation period, Period I (P I), which had a 15 g VS/L feed concentration and a feed ratio (PM:RS=25:75), the total system HRT was 37 d (T/M=12/25 d) and accompanied with manually operating once a day in this stage. Operation in 126-175 days was the second operation period, Period II (P II), which had a higher feed concentration: 20 g VS/L, which was also the target concentration in this study, and fixed feed ratio, the total system HRT was reduced from 37 to 27 d (T/M=9/18 d). The operation mode was still kept manually once a day.

Operation in 176-306 days was the third operation period, Period III (P III), which only had an adjustment in HRT from 27 to 20 days (T/M=6/14 d). Furthermore, in addition to the operating adjustments, the TPAD system had a significant change at day 200 which changed the operation of decanting and feeding from batch manual mode to the semi-continuous automatic pumping mode, of course, problems were resulted from a large extent of change in the device configuration. So to successfully decanting and feeding, the ratio of PM and RS must be adjusted, from the ratio of PM:RS=25:75 in the beginning to the ratio of PM:RS=30:70, PM:RS=50:50 and PM:RS=80:20 at day 269, 283 and 286, respectively. Although the last change of the ratio had an effective improvement in pumps working, reactors had serious foaming, and the performances were unstable. Foaming problem was controlled after ten days operating but still needed more time to let reactors stabilize. Operation from day 307 until the first sampling of pseudo steady-state data was the fourth operation period, Period IV (P IV), HRT in this period reached the goal (14 d of system, T/M=4/10 d). And after about 170 days operating, TPAD system was carried out the first sampling of pseudo steady-state data (Pseudo steady-state I, PSS I) as well as after another 80 days operating, TPAD

system was carried out the second sampling of pseudo-steady-state data (Pseudo steady-state II, PSS II).

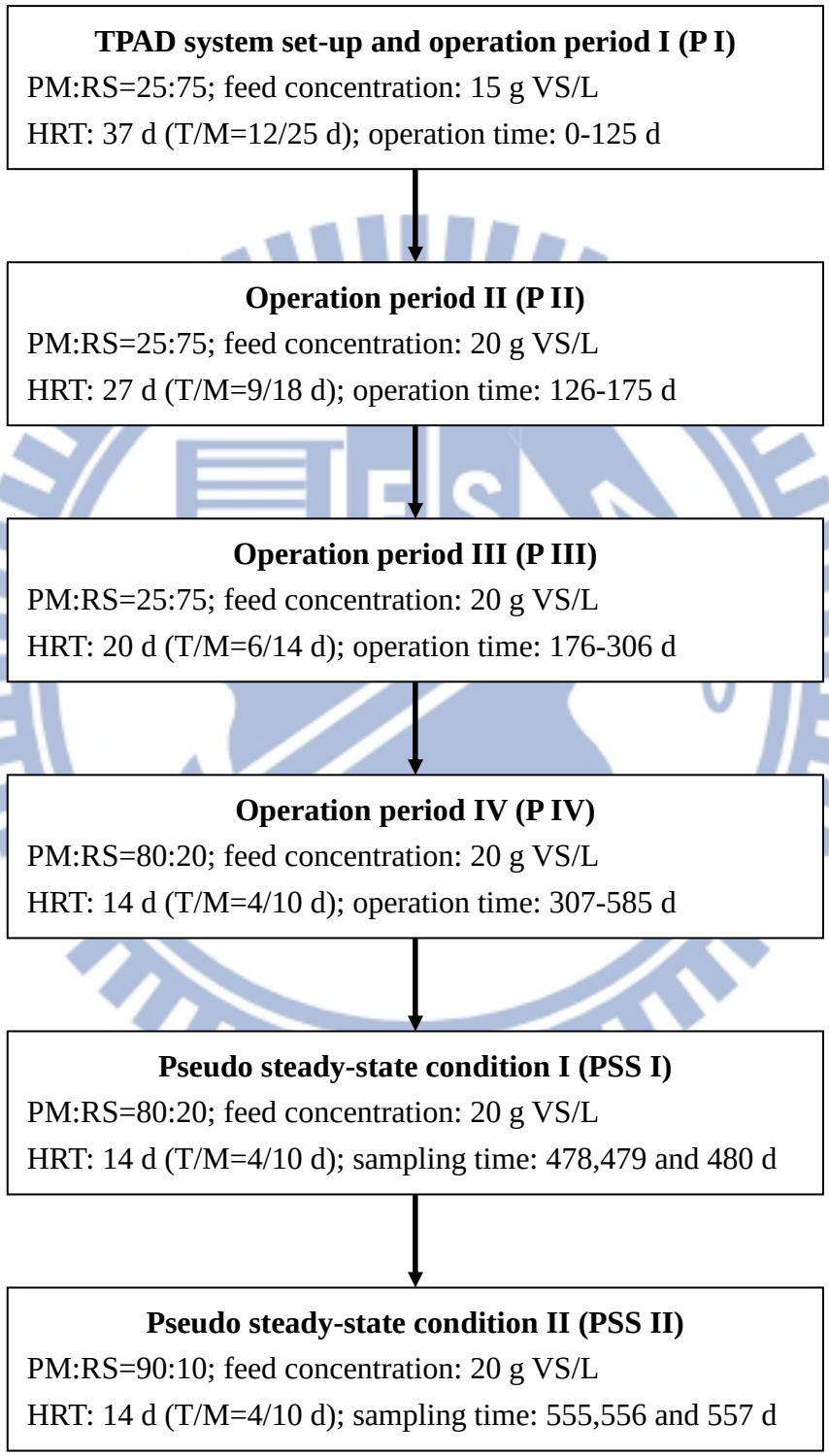


Fig. 3-1 Experimental flowchart

The configuration of TPAD system is like Fig. 2-6, constituted by five tanks: feed tank, thermophilic reactor, thermophilic effluent tank, mesophilic reactor and final effluent tank. And Fig. 3-2 is the photo of TPAD system. The working volumes of thermophilic reactor and mesophilic reactor are 12 L and 20 L, respectively, and both reactor were kept at the selected temperature, which was 55±1 ℃ in thermophilic stage and 35±1℃ in mesophilic stage, by water circulating in the water jacket of the reactors.

Fig. 3-2 The photo of TPAD system
A Feed tank; B Thermophilic reactor; C Thermophilic effluent tank;
D Mesophilic reactor; E Final effluent tank; F Water bath; G Gas meter

The operation mode was semi-continuous which was controlled the apparatuses of decanting, feeding and mixing by timer. Both reactor were decanted and fed at 6-hour intervals and before beginning of feed, the decanting pump must first discharge the digestate to avoid short-circuiting. Each decanting and feeding was

not more than 30 seconds, and the two reactors were mechanically stirred 10-minute at 30-minute intervals, all procedures of decanting and feeding were accompanied with mixing to ensure that the homogeneity of the sludge and substrate. The feed tank and thermophilic effluent tank were stored at 4℃ refrigerator, the final effluent tank was stored at ambient temperature.

The biogas collection equipments, which consisted of a gas balance ball, a gas observation bottle, a hydrogen sulfide scrubber, and a wet gas meter. Function of the gas balance ball was used supplement to the loss of gas volume resulting from discharging the effluent and reduced the surface disturbance. The gas observation bottle was filled with about a quarter of water in a 250 mL serum bottle, the hydrogen sulfide scrubber was filled with steel wool as the scrubbing medium in a 1 L serum bottle and was replaced regularly to avoid corrosion of gas meters. Finally, the wet gas meters recorded the daily gas production, and the exhaust from the gas meter was collected by gas sampling bags.

The operation in the whole accumulation period was gradually reduced the system HRT to achieve the optimum HRT. The initial total HRT was 37 days (T/M= 12/25), the first total HRT was reduced in day 126, decreased from 37 to 27 days (T/M=9/18), the second was reduced in day 176, decreased from 27 to 20 days (T/M=6/14), and the final reduction was implemented in day 307, decreased from 20 to 14 days (T/M=4/10), which was the goal of HRT in this study. In addition, the initial concentration of feed mixed with PM and RS was 15 g VS/L and further increased to 20 g VS/L in day 126, which was the goal of VS concentration and maintaining this level until the end of the experiment.

The TPAD system was operated at batch mode and manually feeding and decanting from day 1 to day 199, and this system was changed the operation from batch mode to semi-continuous mode in day 200. The alteration in operation

inevitably deteriorated the stability of system, moreover the mixture was also radically changed the PM and RS ratio from 25:75 (PM:RS) to 80:20 (PM:RS) to reduce obstruction in the pumps or the pipes. These change both operation and feed composition were significantly affected the performances and thus lengthened the accumulation time.

After nearly 200 days of accumulation facilitated microorganisms to adapt new ratio of feed and both thermophilic and mesophilic reactors reached the pseudo-steady-state, TPAD system was continuously sampled at day 478, 479 and 480 to assess whole performances of the first steady-state (PM:RS=80:20), and once again TPAD system was continuously sampled at day 555, 556 and 557 to evaluate whole performances of the second steady-state (PM:RS=90:10). The pseudo-steady-state condition was defined that parameters such as pH, biogas production, TS, VS, VFAs and alkalinity didn't appear large fluctuations after reactors were continuously operated exceeding the time, which equals three times of the total HRT.

3.2 Experimental runs and assessment of performances

Operation periods and pseudo steady-state conditions of TPAD system were the two emphases in this research including two ratios of PM and RS (PM: RS=80:20 and 90:10). Assessments of the reactor performances were divided into two parts: one was operation in operation periods, which focused on the effluent qualities of thermophilic and mesophilic reactors; the other took the parameters of final mesophilic effluent in pseudo steady-state conditions such as biogas production and composition, VS removal, pathogens reduction, nutrients (N, P and K) and heavy metals as the main considerations. In the operation periods, pH and biogas

production were monitored every day, other parameters like TS/VS, alkalinity and VFAs concentrations were monitored twice a week. Through the change of parameters or not, and then adjusted the reactor operations to the target of retention time (4-day in thermophilic reactor and 10-day in mesophilic reactor) or the target of feed VS concentration (20 g VS/L).

Daily pH monitoring ensured reactors maintained anaerobic microorganisms in the proper growth range, if pH dropped to 6.8 or continuously declined, an additional alkalinity using sodium bicarbonate was necessary. Daily biogas production monitoring could be judged the operation status and also be used as the change of operation according to the production fluctuation. The amount of biogas relates to the removal of organic matters, except some used as cell synthesis, the majority of organic matters are converted to methane and carbon dioxide, and therefore volatile solids removal can be regarded as a factor assessing the reactor condition.

Organic compounds are decomposed by hydrolytic and fermentative bacteria and then are transformed to VFAs, but too much acid will cause the pH dropped to a detrimental range for biomethanation, thus monitoring the VFAs and alkalinity concentration still need to do. Usually the formation of high VFAs concentration is caused by acetate however, many studies have pointed out that propionate is the main reason causing VFAs accumulation in thermophilic AD. Alkalinity is approximately 5000 mg $CaCO_3$/L in regular mesophilic operation, this range can effectively prevents obvious pH drop, but it must note the alkalinity depletion when reactor loading rate is increasing. Compared with mesophilic AD, the alkalinity of thermophilic AD is lower, so it should pay more attention to the changes of VFAs and alkalinity, an extra alkalinity is need if necessary.

Thermophilic methanogens can withstand a greater load than mesophilic

anaerobes due to an accelerating metabolic activity in high temperature environment, but a higher decay rate is simultaneously accompanied with thermophilic methanogens. On the other hand, if a lower loading rate is accepted that may cause the decay rate higher than the growth rate of thermophilic methanogens, and finally result in the reactor operation failed. Therefore, operation of thermophilic reactor is more time-consuming and unstable, especially in the start-up period that thermophilic methanogens aren't sufficient to achieve a safe range. Besides, parameters of operation that dramatically change in feed concentration, composition and retention time or the equipment problems will extend the operation period due to needing a longer time for thermophilic methanogens to again reach the steady state.

The most important purpose evaluates the feasibility that is co-digestion of PM and RS to produce biofertilizer by TPAD system, so there are three aspects needing to consider the effluent from TPAD system if it uses as the biofertilizer which are sanitation, nutrients and harmful ingredients. The assessment of sanitation is according to detection of Coliform group, this is also one of the main quality standards for biofertilizer in Taiwan. Nitrogen, phosphorus and potassium are considerations that are the main evaluation of nutrients not only for chemical fertilizers but also for biofertilizers. The nitrogen detection of this research involved with total Kjeldahl nitrogen (TKN) and ammonium concentration, TKN is the sum of organic nitrogen and inorganic ammonium. In the anoxic or anaerobic status, organic nitrogen is primarily converted to ammonium via ammonification therefore, the concentrations of nitrite and nitrate transformed via nitrification which needs oxygen to participate in the reaction are too low to detect them and as a result, nitrite and nitrate could be ignored in this study. Inorganic ammonium is favorable for plants uptake rather than organic nitrogen, so the ammonification in anaerobic

process should keep in mind. Total phosphorus (TP) and orthophosphate are two major assessments on the detection of phosphorus, and the method of detection of potassium is the same detection of heavy metals using pre-treatment acid digestion of samples and then detecting the metal concentrations through flame atomic absorption spectrophotometer (FLAA).

Compared with industrial wastes, livestock waste and agro-waste don't have a lot of harmful ingredients, but it still notes the type of harmful ingredients which are common may be the excess heavy metals especially in swine manure. The concentrations of copper and zinc often have a very high level than other metals in swine manure due to the considerations of pigs' growth and disinfection, thus these two metals may exceed the control concentrations by regulations. Other metals up to detrimental level are probably negligible.

3.3 Pig manure and rice straw: sources and characteristics

The mesophilic inoculum sludge was provided from a piggery in Miaoli County, Taiwan. The thermophilic inoculum sludge used the same raw sludge 12 L but was directly domesticated at 55±1℃ without dilution in the reactor, on the other hand, the mesophilic inoculum sludge also used the same inoculum but was diluted to one third of the reactor volume with deionized water (DI water) moreover, both reactors didn't further feed the substrate after seeding. Tab. 3-1 is about some features of raw inoculum sludge, pig manure and rice straw.

The PM was collected from another private piggery in Miaoli County, Taiwan. This piggery breeds approximately 9500 pigs with various growth stages and types, and all of them are bred with fodder. Like most piggeries in Taiwan, this piggery applied a three-step wastewater treatment, which includes solid-liquid separation by

screening method, anaerobic treatment, and aerobic treatment (Tsai and Lin, 2009).

Tab. 3-1 Characteristics analyses of pig manure, rice straw and inoculum sludge

	Pig manure (PM)	Rice straw (RS)	Inoculum sludge
pH	7.08	—	7.16
TS (g/L)	108.43	81.30	49.55
VS (g/L)	93.06	70.41	29.39
Alkalinity (mg $CaCO_3$/L)	5125	—	—
TKN (mg N/g DM [a])	21.66	7.13	—
NH_4^+-N (mg N/L)	1029	—	—
TP (mg P/L in PM) (mg P/g DM in RS)	178.60	1.10	—
PO_4^{3-}-P (mg P/L)	12.18	—	—
K (g/kg DM)	2.90	14.4	—
Cd (mg/kg DM)	ND [b]	0.6	—
Cr (mg/kg DM)	18.2	16.0	—
Cu (mg/kg DM)	56	3.4	—
Ni (mg/kg DM)	10.6	9.6	—
Pb (mg/kg DM)	1.0	1.2	—
Zn (mg/kg DM)	234	31.0	—
[c] Cellulose (%)	—	38	—
[c]Hemicellulose (%)	—	35	—
[c]Lignin (%)	—	7	—

[a] Dry matter

[b] Not detected

[c] Data from 陳怡君 (2011)

In order to avoid the lack of PM solid concentration and nutrients, the PM using in this study was collected from equalization pond, which is installed before solid-liquid separation step, and the raw PM was refrigerated at 4℃ darkroom to ensure its freshness. The composition of PM is affected by many factors, for example fodder, growth additives, types and growth stage of pigs etc. 蘇天明等 (2009) indicated the amount of PM was influenced by weights and the feed intake,

in addition the daily emissions of nitrogen, phosphorus, copper, zinc, BOD, and COD would rise with increase of a pig's weight.

The RS was supplied from Agriculture Department of Hsinchu County Government, also produced in Hsinchu County. From Tab. 3-1, we can see the straw is mainly composed of cellulose, hemicellulose and lignin. Lignin is difficult to decompose in anaerobic processes because of natural cellulose in its formation accompanies with the formation of lignin, the protection causing from lignin increases the difficulty of interaction between cellulose and microbial enzymes (賀延齡, 1998).

3.4 Analytic methods

The analytic experiments were divided into two parts, which included the monitoring data in accumulation periods and the TPAD performances data in pseudo-steady-state conditions. Assessments of analytic experiments in accumulation periods mainly had pH, biogas production, TS, VS, alkalinity and VFAs concentration, and the analytic experiments in pseudo-steady-state conditions not only included experiments in accumulation periods but still had biogas composition, VFAs composition, TKN, ammonium, FA, TP, phosphate, detection of coliform group, heavy metals and potassium.

All above-mentioned experiments were carried out in accordance with the standard method announced by A.P.H.A. (1998). Furthermore, for the sake of quick and convenient measurement at VFAs concentration, we used a titration method according to Anderson and Yang (1992). At the analysis frequency, pH and biogas production were monitored daily, TS, VS, alkalinity and VFAs concentration were monitored twice a week in accumulation periods, and all experiments were

monitored 3-day continuously in pseudo-steady-state conditions.

3.4.1 Operation periods

Due to thermophilic anaerobes need more time to adapt if using mesophilic sludge as the inoculum, thus TPAD system had to be monitored the performances of reactors to ensure it at stable status without severe problems. Both of thermophilic and mesophilic reactors were monitored pH by a pH meter, and should carry out the pH 4 and 7 two-point calibration before starting of measuring, biogas productions of two reactors were monitored by wet gas meters. Either pH or biogas production were measured on site, others experiments were conducted in the laboratory. Each sampling was achieved by directly using graduated cylinder to ensure the freshness of samples.

At solids analysis, took 10 mL unacidified mixed samples into the known weight of the evaporating dish and placed in 105℃ oven overnight, and then moved to the dryer to cool 30 min, again measured the weight of sample and dish, as a result, got the TS concentration after calculation. After measuring the weight of sample and dish, put it in the 550℃ high-temperature furnace one hour, once again cooled in oven 10 min, dried 30 min and measured the weight, got the VS concentration after calculation.

The experiment of alkalinity concentration was carried out by a titration method, and the measurement of alkalinity was mainly depended on total alkalinity. The experimental procedure was first, determined the pH of unacidified mixed samples and then samples were titrated by 1 N H_2SO_4 to the end-point of pH=4.5. The consumption of acid volume after calculated is the concentration of total alkalinity.

Except using gas chromatograph (GC) to get the total VFAs concentration,

another method, which was a titration method and was developed from Anderson and Yang (1992), was more convenient and fast to obtain the total VFAs concentration as well as bicarbonate concentration. Experimental procedure was as the following: determined the pH of unacidified mixed samples and then samples were titrated by 1 N H_2SO_4 to the first end-point of pH=5.1 and the second end-point of pH=3.5, calculated the consumption of acid volume of two titration individual end point and would get the total VFAs concentration. Because of similar experimental procedures in determination of total alkalinity and total VFAs concentrations, two experiments could achieve at the same time just recording the consumption of acid volume at different end-points.

3.4.2 Pseudo steady-state

The experiment of biogas composition was determined by the method of gas chromatography-thermal conductivity detector (GC-TCD); this method needed to establish a calibration curve using a standard gas and thereafter, the peaks produced from samples were converted to the volume percentage of biogas composition through the calibration equations. Conditions of GC-TCD were set as following: injector temperature: 80℃; oven temperature: 120℃; detector temperature: 180℃; helium was the carrier; the flow rate used was 20-30 mL/min. Each injection volume was 1 mL and the each acquisition time was 20 min, all samples were carried out three replicate analyses. The composition of standard gas was 70% CH_4, 25% CO_2 and 5% N_2 (by volume). All gas samples were measured immediately at the sampling day to avoid analysis errors resulting from the collection and transportation.

The experiment of VFAs composition was determined by the method of gas chromatography-flame ion detector (GC-FID); like GC-TCD, this method also

needed to establish a calibration curve using a volatile acid standard mix and then converted by the calibration equations to get individual concentrations of VFAs. Conditions of GC-FID were set as following: mode was separation and the separation ratio of water was 10:1; control mode was flow; the equilibration time of oven: 0.5 min; the recommendations of the initial temperature of VFAs: 60℃ and the duration time: 1 min; heating rate: 18℃/min; final temperature: 230℃ maintaining 5 min; detector temperature: 250℃; helium was the carrier. The volatile acid standard mix was composed by acetic, propionic, and butyric acid. Due to a special modification on the chromatography column, both the standard acid and samples didn't require further extraction with organic solvents, and samples could be directly injected into GC-FID, but each injection volume was only 0.4 μL. All samples would be treated by acidification using conc. H_2SO_4 to maintain the undissociated state of VFAs, and all analyses were completed within three days.

Detections of TKN and ammonium were conducted by using digestion and distillation instruments, the difference between two experiments was ammonium analysis didn't need digestion just distilled samples, however, unlike ammonium analysis, TKN required the two steps of acid digestion and distillation. In the step of acid digestion, took a 10 mL sample and 10 mL conc. H_2SO_4 into the digestive tube and added one or two digestive pills, which contain potassium sulfate and selenium, and then put the tube into the 400℃ heating device at least one hour, the digestion time depended on the difficulty of sample digestion. After digestion and 30 min cooling, adding 100 mL DI water into this tube and distilled the mix 3-5 min and then got the TKN or ammonium concentrations. Samples could be added conc. H_2SO_4 to pH <3 and saved seven days for ammonium analysis or up to 14 days for TKN analysis at 4℃.

Experiments of TP and phosphate were used spectrophotometric method.

Like TKN analysis, TP experiment also need acid digestion converting organic P into phosphate and detected the amount of phosphate to get TP concentration. A 50 mL acidified mixed sample was added with 1 mL 11 N H_2SO_4 and 0.4 g ammonium persulfate and was placed in a 250 mL serum bottle. And this mixture was put in an autoclave set the condition at 120℃ and 1.0-1.4kg/m^2 and heated 30 min. And then adjusted the mixture's pH to pH=7.0±0.2 using 1 N or appropriate concentration of NaOH solution, and diluted it to 100 mL. Finally, read the absorbance values at nm 880 wavelength from Spectrophotometer and obtained the TP concentration. It must be noted in the calculation of TP is due to dilution, so final concentration needed to be multiplied by twice is the true TP concentration. The phosphate analysis was to only take the part of supernatant liquid of unacidified samples and detected the absorbance values at the same wavelength. Phosphate analysis had to be completed in two days because this experiment was used unacidified samples, but TP analysis could keep up to seven days at 4℃.

The assessment of pathogens in this study was completed by using the membrane filtration method of coliform group. Except sampling, the whole procedure of experiment was carried out by a certified contract laboratory (亞太環境科技公司). The procedure of sampling was: took supernatant liquid of the sample and then packed it into 100 mL sterile sampling bags and last, samples were transported at 4-8℃ to the laboratory to complete follow-up experiments.

The detection procedure of potassium was the same detection of heavy metals, and these experiments were also carried out by certified contract laboratories (惠民實業公司, 亞太環境科技公司). Due to samples contained high concentrations of solids, so samples must need acid digestion pre-treatment and then could be conducted follow-up metal experiments, the digestion procedure was described below.

First, the sample placed in 105℃ oven until the moisture was evaporated and then took the dry sample 0.5-1.0 g into 250 mL glass beaker. Second, added 10 mL (1+1) HNO_3 and covered with a watch glass and put the sample in 95±5℃ hot plate 10-15 min, making certain that sample did not boil. Cooled the sample 5 min and then added 5 mL conc. HNO_3, again heated at 95±5℃, if the sample appeared brown smoke showing it was oxidizing, repeated these reflux heating and cooling steps until the smoke disappeared. Maintained at 95±5℃ two hours and kept the sample without boiling.

Third, after cooling, added 2 mL DI water and 3 mL 30% H_2O_2 covered with a watch glass. Keeping the sample was at a slow heating condition to avoid presence of intense bubbling until the bubbling subsided, and then cooled it. Fourth, each time adding 1 mL of 30% H_2O_2 and kept heating until no longer change of the sample. It must be noted that the total volume of 30% H_2O_2 should not more than 10 mL. Once again, maintained at 95±5℃ two hours and kept the sample without boiling. Finally, due to the instruments of the two certified contract laboratories were FLAA, so the above experimental procedures needed an additional step. Added 10 mL conc. HCl into the digestive liquid and covered with a watch glass, heated this mixture 15 min at 95± 5℃ and avoided boiling. After centrifugation and dilution to 100 mL, the sample could be analyzed the concentrations of heavy metals and potassium by FLAA.

Chapter 4 Results and discussion

4.1 The daily performance of pH and biogas production during operation periods

Fig. 4-1 is the daily performance of pH and biogas production of thermophilic and mesophilic reactors in 585 days operation. In P I (0-125 d), the initial pH range about 6.90-7.00, both reactors were not significantly different even if the mesophilic seeding sludge had been diluted three times. The thermophilic pH had a downward trend after operating 28 days, the minimum pH was 6.78, but the mesophilic pH kept at 6.90. It indicated that high temperature (55℃) environment caused a large number of deaths on the anaerobes of mesophilic seeding sludge, simultaneously, the population of thermophilic methanogens was still less than a certain level. In the report of Boušková et al. (2005), which compared with two heating modes to the impact of domesticating mesophilic sludge at thermophilic condition also had a similar result. They found when temperature rose from 42 to 47℃, the biogas production reduced as well as the methane content, besides, the reactor also appeared a significant increase in VFAs concentrations, especially in acetate and propionate. So from this result, mesophilic methanogens had a large impact on rising temperature, thus insufficient methanogens couldn't quickly consume the VFAs and caused an increase on surplus VFAs. The reduction of thermophilic pH had an improvement after operating 5-7 days, pH backed up to neutral range. However the change of mesophilic pH didn't look as fast as thermophilic pH, the pH range kept at 6.90-6.95 until d 80, and then pH rose to 7.00-7.10 maintain about 20 days and once again rose to 7.40 until the end of P I. Due to the whole mesophilic environment without large fluctuations, an adequate

HRT in mesophilic stage, keeping low concentrations of VFAs as well as sufficient alkalinity, the pH of mesophilic reactor didn't appear any significant negative effects.

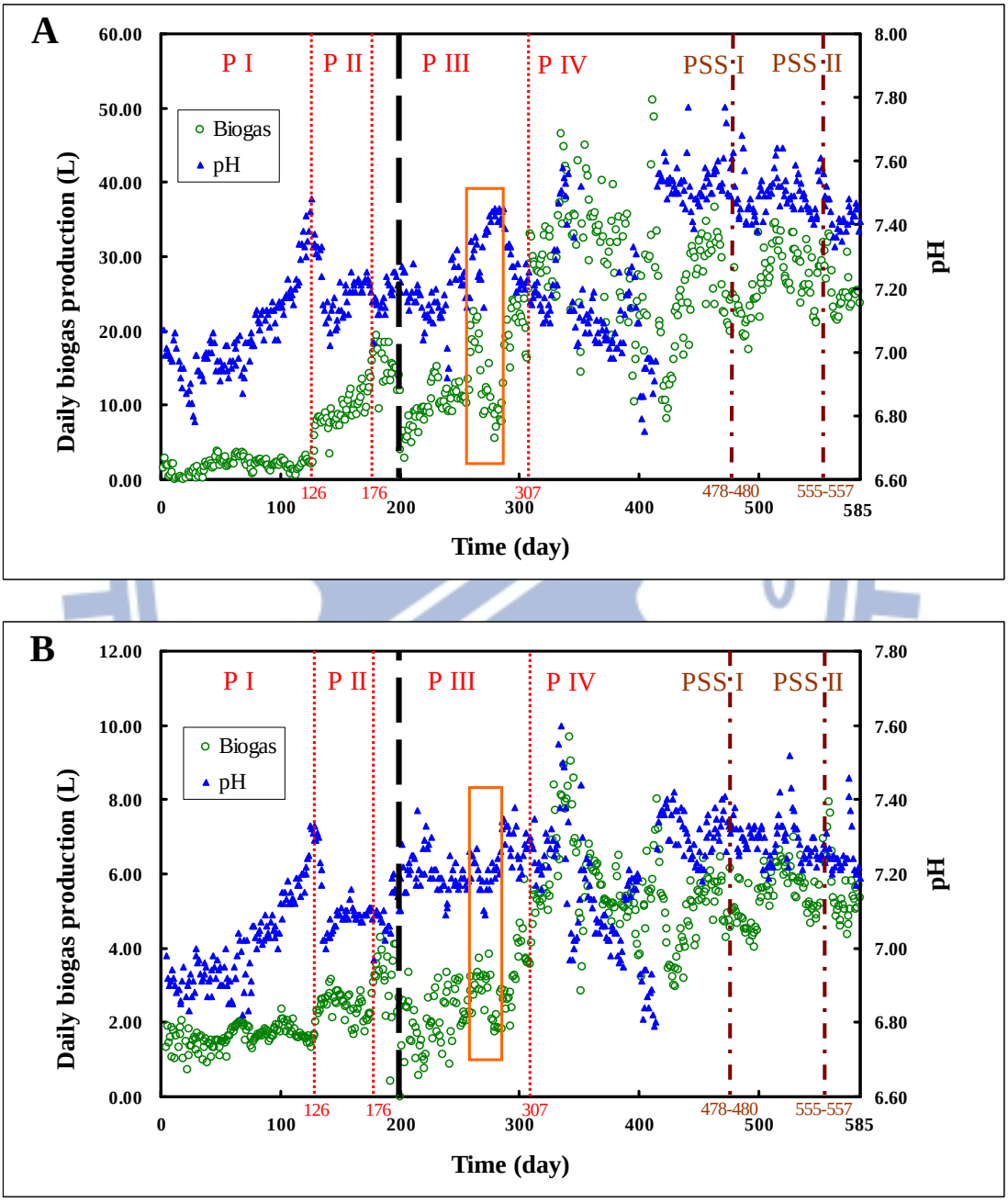


Fig. 4-1 The daily performance of pH and biogas production of (A) thermophilic and (B) mesophilic reactors The gas productions were corrected in the STP condition (0 ℃, 1 atm).

Thermophilic AD had a considerable production than mesophilic AD at daily biogas production from literatures due to a large capacity of loading rate on thermophilic AD, but it didn't had a clear difference on the biogas production at beginning of the operation period. The reason for addition to thermophilic anaerobes was not yet abundant, low solids concentration and long retention time also limited the thermophilic loading rate upgrading. The biogas production of thermophilic reactor gradually declined in the previous 25 days because the majority of mesophilic methanogens died, and the reactor was at quite unstable state, the production reduced from 3 L/d to 0.1 L/d, almost no biogas production. As the pH increased in about 35 days after operating, the biogas production also had an rise at this time. The daily thermophilic biogas production was at range of 1.5-3.0 L/d from d 35 to the end of P I; on the contrary, the change of daily mesophilic biogas production was not as large as thermophilic reactor, the biogas production of second stage in P I was about 1.5-2.0 L/d.

In P II (126-175 d), except reducing system HRT to 27 d, the feed concentration was also adjusted from 15 to 20 g VS/L. Due to a higher loading rate in this period, both of thermophilic and mesophilic reactor declined not only in the daily pH and biogas production but in the alkalinity and VFAs concentration. The thermophilic pH reduced from pH=7.48 at d 126 to pH=7.02 at d 141, the pH didn't deteriorate further, however such a substantial decline in the daily pH associated with a large amount of VFAs concentration. In addition, the alkalinity in thermophilic reactor showed a downward trend since the reactor began operating, and even exceeding 100 days operation, it was still at a low concentration range. The mesophilic reactor was also affected by this operational adjustment, the pH still was 7.33 at d 129, but decreasing to 7.00 at d 138, unlike the thermophilic stage, there was no significant increased in the VFAs concentration only the alkalinity during P II

dropped to the lowest point, which was also the lowest concentration of alkalinity in the mesophilic stage. Two reactors appeared favorable signs after adapting the change of operation, both of pH in thermophilic and mesophilic reactors daily rose during d 140-150 and then maintaining at pH=7.20 at thermophilic stage as well as at pH=7.10 at mesophilic stage until the end of P II (d 176).

Two reactors had conspicuous improvements in daily biogas productions in this period, particularly the thermophilic reactor, and the biogas production increased from 2.1 L/d at d 126 to 14.0 L/d at d 175 before the end of P II. The most important factor is due to the enhancement of the system load, and the population of thermophilic methanogens was more bountiful after more than 100 days domestication. It needs to raise the loading rate by increasing the feed solid concentration or reducing the HRT when the population of thermophilic anaerobes is toward a sufficient level. Because of high temperature environment accelerating microbial growth and a high decay rate of thermophilic methanogens, an insufficient loading rate limits the development of thermophilic methanogens, moreover, it must obviously affect the amount of thermophilic methanogens when the decay rate is larger than growth rate due to a cumulative inhibition of VFAs. That is why most studies recommended that the HRT of thermophilic AD favors at three or four days, and that makes thermophilic AD to be more competitive than traditional mesophilic AD. Although there was no dramatic increase in mesophilic biogas production, it still had an improvement from 1.5-2.0 L/d in P I to 2.0-2.5 L/d P II compared with production in P I. Because most solids were converted to biogas in the first stage, it was foreseeable that biogas production in the mesophilic stage was less than thermosphilic stage. The biogas production of mesophilic stage slightly fluctuated after d 157, indicating that concerned with operational factors. The main function of mesophilic stage in TPAD system is the final effluent polishing. Although

thermophilic AD has some superior performances at VS removal, pathogens reduction as well as a large amount of biogas, the effluent from thermophilic reactor generally accompanies with some shortcomings like high concentration VFAs thus producing odors and poor stability thus varying on the quality of thermophilic effluent. As a result, installation of mesophilic reactor is to overcome some drawbacks of thermophilic AD and stabilize the quality of final effluent. Therefore, biogas production and VS removal are not the main concerns in this stage.

TPAD system started the P III at d 176; the performances of reactors could be expected that mainly impacted on the change of loading rate in this period before d 200. Adjustment of operating parameters is affected on pH and biogas production but is not necessarily simultaneous. This might be related to different metabolic behaviors and growth characteristics between fermentative bacteria and methanogens. It was a most significant impact on TPAD system to change system configuration in a large degree at d 200. The bold dotted line in Fig. 4-1 represented the replacement of reactors and change of the operation mode from batch to semi-continuous. Besides, the significant change of the ratio of PM and RS was also the main reason causing system at an unstable state, the rectangular box in Fig. 4-1 represented the days changing the ratio of PM and RS (PM:RS=30:70 at d269; PM:RS=50:50 at d 283; and PM:RS=80:20 at day 286), no wonder we must again extend the operation time. The thermophilic pH didn't affected immediately, it was still at the pH=7.20 range and then began to decline gradually, it declined to the lowest point, pH = 6.92. The mesophilic pH showed a trend of rise at first and then fall, but generally pH fluctuated at the range of 7.20.

Biogas productions at this time had significant impacts in the beginning of replacement, the dramatic decline in two reactors resulted from the spread when changing the reactors and pumps. After changing the installations, the

thermophilic biogas production rebounded but still unable to reach the production of P II level, on the other hand, the mesophilic biogas production had been very unstable since the change of installations even though the production returned to the original level. This suggested that the thermophilic stage was responsible for the conversion of organic solids, so the thermophilic biogas production could return quickly due to operating in a high loading rate. However, the mesophilic stage in TPAD system was operated at a low loading rate, thus the production was quite low compared with the thermophilic reactor, once the operation significantly changed, the impact would be very obvious.

At d 307, TPAD system entered the last operation period, P IV, which was operated at the goal HRT (T/M=4/10 d). System had been at unstable state since the replacement of the installations, the improvement was not well even though extended the operation time. Of course the quality of each batch pig manure waste might be also the reason causing reactors at unstable condition. The pH both of two reactors changed severely during d 307-400. Generally, thermophilic and mesophilic reactors showed four changes in this period: first, the thermophilic pH increased at d 320-336; the mesophilic pH also increased at d 318-335. Second, the thermophilic pH decreased at d 337-379; the mesophilic pH decreased at d 336-387. And then, the thermophilic pH rebounded at d 380-395; the mesophilic pH rebounded at d 388-400. Finally, once again both reactors' pH decreased at d 396-405 in the thermophilic reactor and at d 401-413 in the mesophilic reactor, respectively. Like change of pH, the biogas productions were also quite unstable, the maximum production was 46 L/d, but the minimum production was only 8.2 L/d in thermophilic stage, on the other hand, the maximum production was about 10 L/d, the minimum production was 3.0 L/d in mesophilic stage.

In other to the fluctuations of pH and biogas production were too large and

resulted in delaying the sampling of pseudo-steady-state, a extra 3 g/L $NaHCO_3$ would add in the feed to supply the thermophilic alkalinity from d 407 until the end of experiment. Due to d 420-585 were relatively stable compared with the previous operation, TPAD system was carried out the first sampling of pseudo-steady-state at d 478-480 (PSS I) and the second sampling at d 555-557 (PSS II), respectively.

4.2 The daily performance of alkalinity and VFAs concentrations during operation periods

Fig. 4-2 is the daily performance of alkalinity and VFAs concentration of thermophilic and mesophilic reactors in 585 days operation. The changes of alkalinity and VFAs concentration and the changes of pH and biogas production are the two faces of one and affect each other. From the P I, the alkalinity of seeding sludge was at about 5000 mg $CaCO_3$/L, the lower concentration in mesophilic stage might be caused by the dilution of inoculum sludge. The thermophilic alkalinity showed a downward trend after one month operation and the lowest came to 2400 mg $CaCO_3$/L, almost loss of about half the amount. The mesophilic alkalinity also showed a downward trend, but the extent and speed were not severe like the thermophilic reactor, it was at 4300 mg $CaCO_3$/L in the first day and downed to the lowest concentration of P I: 3050 mg $CaCO_3$/L in d 96.

The feed composition had a large contribution to reducing the alkalinity because the majority of composition was RS in P I (PM:RS=25:75). The rich-in carbohydrate wastes such as rice straw provide far less alkalinity than manure wastes which contain a large amount of nitrogen source and then will be transformed to ammonium known as one of alkalinity; in addition, VFAs produced by

fermentative bacteria will further consume the alkalinity. The thermophilic alkalinity maintained at 2500 mg $CaCO_3$/L during d 37-96, either the thermophilic reactor or the mesophilic reactor showed another downward trend from d 100 until the end of P I. The instability of TPAD system caused a very high VFAs concentration found both in the two reactors. The high VFAs concentration in thermophilic stage reduced gradually with the increase in accumulation time, the thermophilic VFAs concentration kept at the range of 100 mg acetic acid/L, occasionally increasing concentration occurred. The mesophilic VFAs concentration wouldn't increase until in d 23, the highest concentration was about 2400 mg acetic acid/L. After d 75, the mesophilic VFAs concentration also kept at the range of 100 mg acetic acid/L.

The performances in P II was more stable compared with P II, the thermophilic alkalinity increased from 2200 mg $CaCO_3$/L in d 138 to 3200 mg $CaCO_3$/L in d 171, on the other hand, the mesophilic alkalinity showed a slight decrease at the beginning but it maintained approximately at the range of 3200 mg $CaCO_3$/L. VFAs concentration in thermophilic stage was still unstable, the concentration was about at 150-1000 mg acetic acid/L, the impact of high concentrations of VFAs was also affected the thermophilic pH. VFAs concentration in mesophilic stage still maintained at a low level range approximately 100 mg acetic acid/L.

Replacement of installations and adjustment feed ratios also influenced on alkalinity and VFAs concentration in P III. The disturbance in thermophilic stage was obvious, and a clear distinction in thermophilic alkalinity was found. The thermophilic alkalinity was at the range of 2500-3000 mg $CaCO_3$/L before d 250 and then significantly came to the highest concentration which was approximately at 5000 mg $CaCO_3$/L. A high concentration of alkalinity was supplied from the increase ratio of pig manure, although the risk that caused pH drop rapidly was

reduced, VFAs concentration also accumulated at the same time and deteriorated the system performance. The alkalinity in mesophilic stage was fluctuated by the

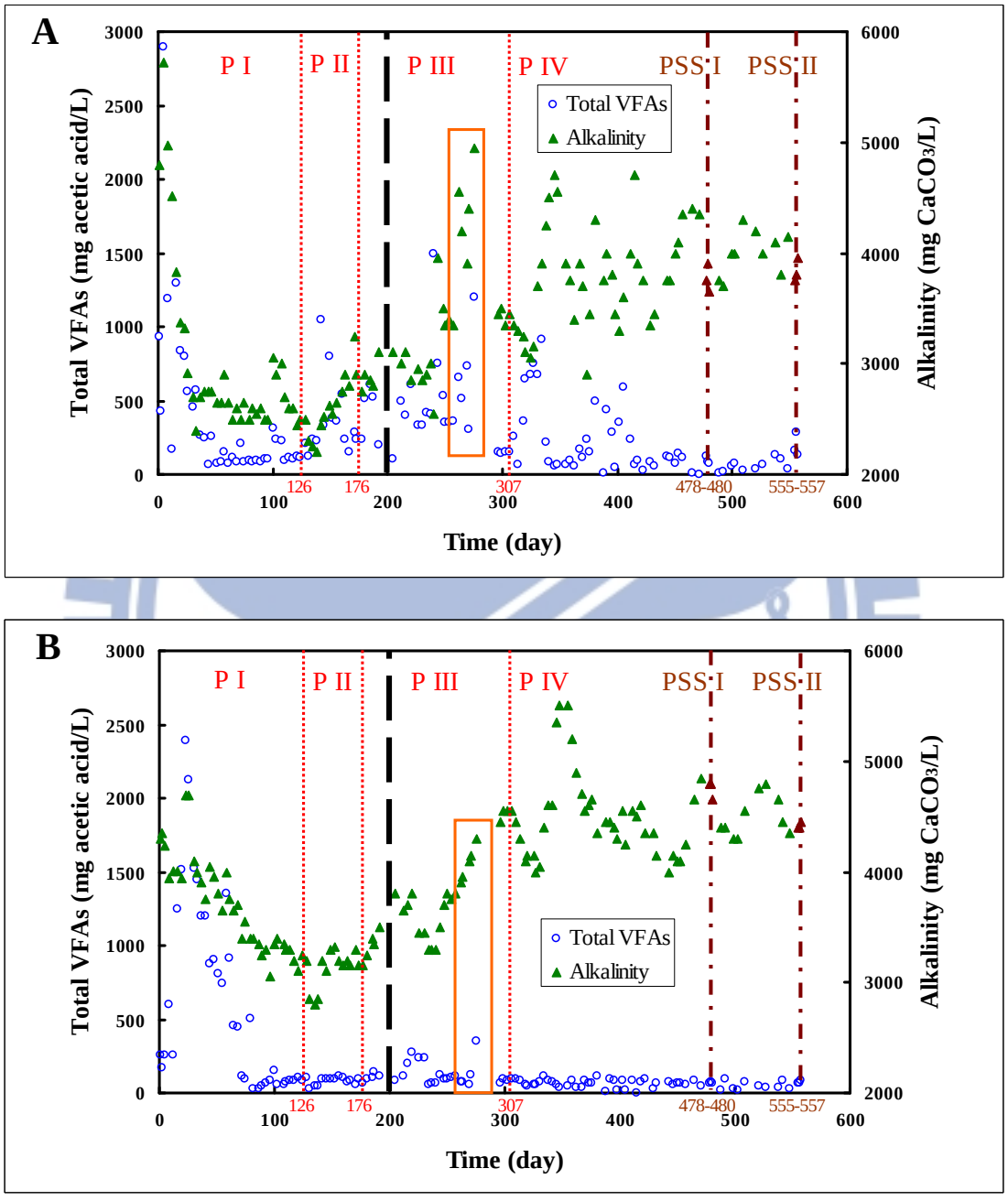


Fig. 4-2 The daily performance of alkalinity and VFAs concentration of (A) thermophilic and (B) mesophilic reactors

change of installations and feed ratios, too. Due to the influent from the first stage containing higher concentration of organic nitrogen and ammonium, it made the

mesophilic alkalinity increase like the change in the thermophilic stage. In the change of VFAs concentration in mesophilic stage and before the end of the experiment, it still didn't exceed 500 mg acetic acid/L even though TPAD system was confronted with some accidents in this period.

The fluctuations of alkalinity and VFAs concentration in thermophilic and mesophilic stage were still highly variable in P IV. Apart form replacement of installations and adjustment feed ratios in the previous period, these fluctuations might be also related to the problem of thermophilic water bath equipment during this period or caused by the different batches pig manure. Generally, alkalinity reduction in two reactors during d 307-327 resulted from the decrease of system HRT, and follow-up changes in the alkalinity were related to problems of operation and feed. The VFAs concentration in thermophilic stage was more variable during d 307-400 but it had an effective improvement after two months operation, the thermophilic VFAs concentration was not more than 300 mg acetic acid/L; the mesophilic VFAs concentration was still at fairly steady state and kept at the range of 100 mg acetic acid/L.

4.3 Description of operation problems and improvements

TPAD system in this study had serious problems which were the pumps operating and obstruction caused by high solid concentration influent. Many researches confirmed that TPAD system can be operated at high loading rate, but the improper equipments used in this TPAD system resulted in the experiment was confronted with serious obstruction in the entire operating period. Because system needed to feed four times every day, the accumulation of thermophilic anaerobes and TPAD performances would be adversely affected if the obstruction problem

couldn’t solve immediately. Besides, due to the feed containing RS, it should conduct with physical pre-treatment to avoid obstruction of pumps or connections between pumps and pipes appearing.

Therefore, this experiment with the situation which was unable to replace the pumps and other installations took the following methods to overcome the obstruction problem. The first method was adjustments of solid concentration and feed composition. The feed concentration maintained 15 g VS/L in accumulation period and the maximum feed concentration using in pseudo-steady-state condition was only 20 g VS/L. Besides, three originally expected feed ratios (PM:RS=75:25, PM:RS=50:50 and PM:RS=25:75) were changed to two feed ratios (PM:RS=80:20 and PM:RS=90:10). The second method was changing the operation of thermophilic reactor from four times automatic feed each day to two times manual feed each day. Mesophilic reactor still kept automatic operation due to nonexistent obstruction problem in this stage. The third method was RS carried out destruction of physical pre-treatment before using as the substrate.

4.4 Biogas yield and composition during pseudo steady-state (PSS I and II)

Tab. 4-1 is the biogas productions, yields and compositions of thermophilic and mesophilic reactors of TPAD system in pseudo steady-state conditions. The production had an increasing trend in PSS II compared to PSS I, this might be related to a higher ratio of PM in PSS II. Although the ratios of PM and RS in PSS I and II didn’t have an obvious difference. Besides from the biogas yield, we could see thermophilic stage was the main performance in TPAD system because the thermophilic yield was higher more twice than the mesophilic yield in PSS I. But

thermophilic yield in PSS II was lower than mesophilic yield due to the equipment problems worsening the thermophilic stage. Because the equipment problems caused that thermophilic methanogens couldn't consume VFAs effectively and then mesophilic methanogens were responsible for conversion of these undigested redundant VFAs.

Tab. 4-1 The biogas characteristics during PSS I and II

	PSS I		PSS II	
	Thermo.[a]	Meso.[b]	Thermo.	Meso.
Production (L/d)	25.97±1.77	5.05±0.02	28.79±1.58	6.44±0.07
Yield (L CH_4/g VS_{des})	0.56±0.01	0.27±0.01	0.40±0.07	0.63±0.09
CH_4 (%)	51.93±1.95	55.99±5.06	53.89±2.08	61.82±2.99
CO_2 (%)	28.62±1.95	17.94±1.64	26.92±0.82	28.56±1.35
N_2 (%)	2.10±0.19	4.14±0.04	5.60±0.46	2.88±0.32

[a] Thermophilic stage

[b] Mesophilic stage

There was not much difference between thermophilic and mesophilic stages at the biogas composition. The low methane content in this research might be caused by the poor domestication. In addition like many previous studies, the methane content in mesophilic stage was always higher than thermophilic stage, this reason might be related with the population of anaerobes. Because hydrogen producing bacteria prefer at a thermophilic condition and lead to a higher partial pressure of hydrogen, literatures indicated that hydrogen was detrimental at biomethanation and propionate metabolism even its content was quite low in biogas composition.

The mesophilic methane content in PSS II was higher than thermophilic once again confirmed the problems of installations and operation deteriorated not only in biogas production but in yield. Although the methane content didn't decline significantly, a large amount of methane in mesophilic stage suggested that

thermophilic methanogens were influenced, and the function of mesophilic stage was shifted from polishing effluent to the main biomethanation. As the accumulation of thermophilic methanogens was more difficult, the thermophilic performance would be very hard to return to recovery status if there were any other problems at impaired thermophilic stage. It still needs more researches in the future to investigate the effect of operation changes on microbial dynamics.

4.5 VS removal during pseudo steady-state (PSS I and II)

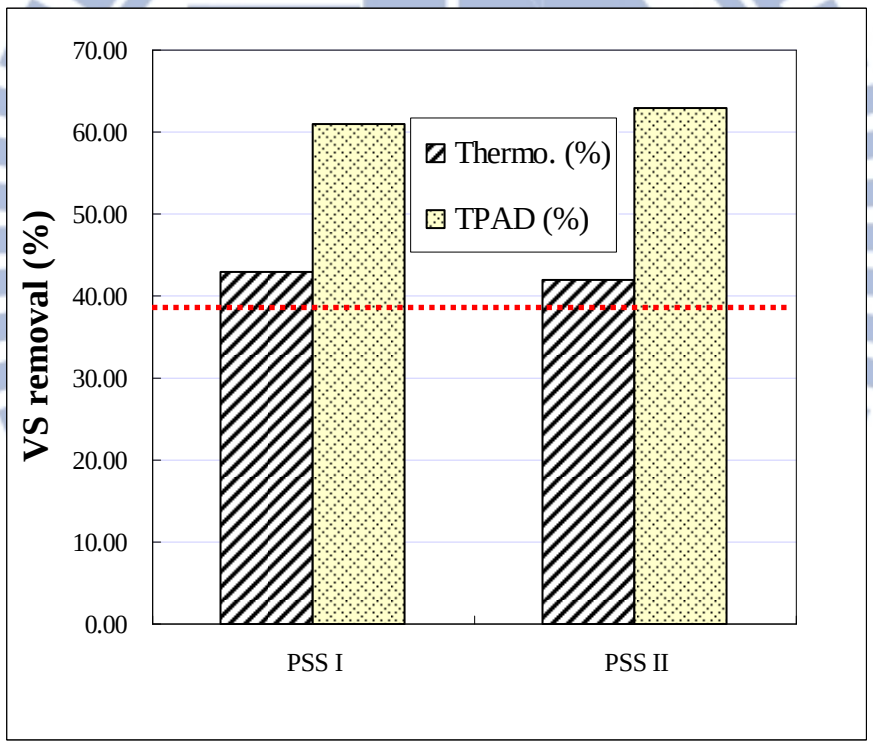


Fig. 4-3 The VS removal of the thermophilic reactor and the entire system during PSS I and II

Fig. 4-3 is the VS removal of the thermophilic reactor and the entire system. Even though there were thermophilic equipment problems in PSS II, the TPAD system both in PSS I and II were still excellent in VS removal, and met the

regulation of Class A biosolids, which stipulates that VS removal should be at above 38% (the dotted line in Fig. 4-3). The feed VS maximum concentration in this study was 20 g VS/L, and loading rate was still at an acceptable range. That was why the VS removal didn't appear significant deterioration in this study. From the results, the VS removal in this TPAD experiment could achieve higher than 60% removal and just in the thermophilic stage could achieve more than 40% removal if without any operation problems. The thermophilic VS removal in PSS II was slightly lower than PSS I, indicating resulted from equipment problems.

4.6 pH, alkalinity and VFAs concentration during pseudo steady-state (PSS I and II)

Tab. 4-2 is some characteristics about pH, alkalinity and ratios of VFAs and alkalinity in two pseudo steady-state conditions. The pH range in PSS I and II were approximately equal to 7.49-7.61 in thermophilic stage and 7.27-7.36 in mesophilic stage, respectively. Thus both of two stages in this TPAD system were suitable for the growth of methanogens. The pH and alkalinity in PSS II were lower than in PSS I, suggesting these were result from a large amount of VFAs and more depletion of alkalinity, but generally pH and alkalinity were still at a safe level due to a lower system OLR used in this study. We could get the information that to determine whether the alkalinity is too low or whether VFAs accumulated occurs from the ratio of VFAs and alkalinity. Sung and Santha (2003) indicated that VFAs/Alkalinity ratios lower than 0.35 in the thermophilic stage and 0.10 in the mesophilic stage are optimal for TPAD system treating cattle manure at high solids concentrations.

Tab. 4-3 is about concentration and composition of VFAs. The concentration

of VFAs in PSS II increased due to equipment and operational problems. Because VFAs accumulation didn't occur even in PSS II, and follow-up mesophilic stage still converted VFAs to methane effectively, as a result, the effluent of VFAs concentration could keep at a low level.

Tab. 4-2 pH, alkalinity and VFAs/Alkalinity ratios of thermophilic and mesophilic stage during PSS I and II

	PSS I		PSS II	
	Thermo.[a]	Meso.[b]	Thermo.	Meso.
pH	7.61±0.02	7.36±0.02	7.49±0.02	7.27±0.03
Alk[c]	3767±103	4750±71	3833±85	4417±24
VFAs/Alk.	0.0212±0.0004	0.0134±0.0001	0.0380±0.0041	0.0160±0.0001

[a] Thermophilic stage
[b] Mesophilic stage
[c] Alkalinity (mg $CaCO_3$/L)

Tab. 4-3 The concentration and composition of VFAs during PSS I and II

	PSS I		PSS II	
	Thermo.[a]	Meso.[b]	Thermo.	Meso.
pH	7.61±0.02	7.36±0.02	7.49±0.02	7.27±0.03
Acetic (mg/L)	13.08±0.30	5.04±0.00	59.41±6.95	15.07±0.30
Propinic (mg/L)	58.23±1.65	ND[d]	69.56±0.35	ND
Butyric (mg/L)	ND	ND	ND	ND
Total VFAs (mg acetic/L)[c]	80.00±4.00	63.50±1.50	146.0±12.00	70.50±0.50

[a] Thermophilic stage
[b] Mesophilic stage
[c] Measured by titration method (Anderson and Yang, 1992)
[d] Not detected

There was a slightly different, especially in the mesophilic stage, between the plus of individual acids and total VFAs concentration due to resulted from different measurement methods. The total VFAs concentration was measured by using a

titration method, however individual acids concentrations was detected by using GC-FID method. Even if no VFAs accumulation occurred, a small amount of acetate was detected both in thermophilic and mesophilic reactors, furthermore propionate was also detected only in thermophilic reactors, but neither in thermophilic nor mesophilc reactors detected any butyrate in PSS I or II.

Propionate is a common organic acid in thermophilic AD and even is a main problem that causes VFAs accumulation. Because H_2 concentration is higher in thermophilic status and it also forces the metabolism of odd-numbered carbon acids to generate the final intermediate propionate instead of acetate. Only H_2 concentration maintains at a very low level to be able to make propionate further transformed to acetate, formate, H_2 and CO_2 via methyl-malonyl-CoA pathway, or another pathway, which makes propionate be condensed to a six-carbon intermediate and then this intermediate is further cleaved to acetate and butyrate (Bryant 1979; de Bok et al., 2004). Therefore, how to keep a quite low H_2 partial pressure in thermophilic reactor is an important operating consideration.

4.7 Nutrients (N, P and K) during pseudo steady-state (PSS I and II)

Tab. 4-4 and 4-5 are the conversion of nitrogen, phosphorus and potassium in PSS I and II. In the part of nitrogen, the TKN concentrations of feed had little difference in two pseudo steady-state conditions. But ammonium concentration was slightly higher in PSS II due to an increase PM ratio in this period. Thermophilic TKN concentrations were always lower than feed or mesophilic reactor might be related with more ammonia emission in a high temperature condition when thermophilic sample was carried out. Besides, from the ratio of

NH_4^+-N/Org.-N, we could see the change of ammonification in thermophilic and mesophilic two-stage.

Tab. 4-4 Levels of nutrients during PSS I

PSS I	Feed	Thermo. [a]	Meso. [b]
pH	7.27±0.05	7.61±0.02	7.36±0.02
TKN (mg N/L)	661.28±25.02	538.82±49.60	615.30±13.95
NH_4^+-N (mg N/L)	81.50±7.47	198.02±10.09	360.80±25.85
NH_4^+-N/Org.-N	0.14	0.58	1.42
Org.-N removal (%)	—	—	56.10
NH_3 (mg N/L)	0.17±0.02	25.74±2.13	8.89±0.37
TP (mg P/L)	106.78±3.69	—	101.11±13.90
PO_4^{3-}-P(mg P/L)	35.28±12.37	—	21.11±2.93
PO_4^{3-}-P removal (%)	—	—	40.16
PO_4^{3-}-P /Org.-P	0.49	—	0.26
N:P [c]	6.19:1	—	6.09:1
K (g/g DM [d])	—	—	4.18±0.16

[a] Thermophilic stage
[b] Mesophilic stage
[c] Calculated on the basis of TKN and TP
[d] Dry matter

Ammonification in mesophilic stage had a significantly larger degree than in thermophilc stage, although thermophilic stage was considered that the main performances such as solids removal, biogas production and pathogens reduction on TPAD system. However it seemed that mesophilic stage played an important role on the performance of ammonification, particularly the TPAD effluent was applied as a biofertilizer or a soil conditioner because inorganic nitrogen is easier absorbed by plants. Organic nitrogen removal could be higher than 50% by using TPAD system if at a good operating mode and an appropriate ratio of PM and RS. NH_3 concentrations were higher in thermophilic reactor, but both of PSS I and II

wouldn't appear NH_3 inhibition due to operating at relatively low OLRs compared with literatures.

Tab. 4-5 Levels of nutrients during PSS II

PSS II	Feed	Thermo. [a]	Meso. [b]
pH	7.10±0.02	7.49±0.02	7.27±0.03
TKN (mg N/L)	660.75±23.08	635.17±23.95	646.33±18.25
NH_4^+-N (mg N/L)	115.47±5.86	225.60±4.89	298.47±43.00
NH_4^+-N/Org.-N	0.21	0.55	0.86
Org.-N removal (%)	—	—	36.20
NH_3 (mg N/L)	0.17±0.02	24.06±0.70	6.08±0.51
TP (mg P/L)	116.80±9.46	—	108.66±5.85
PO_4^{3-}-P(mg P/L)	35.94±12.93	—	22.34±4.83
PO_4 removal (%)	—	—	37.84
PO_4^{3-}-P /Org.-P	0.44	—	0.26
N:P [c]	5.66:1	—	5.95:1
K (g/g DM [d])	—	—	8.95±1.18

[a] Thermophilic stage

[b] Mesophilic stage

[c] Calculated on the basis of TKN and TP

[d] Dry matter

The TP concentration didn't have an obvious removal effect either in PSS I or PSS II, it still had a high level in the final effluent. Compared to a high conversion rate of nitrogen, the conversion for TP to phosphate appeared a downward trend both in PSS I and II, this implied that most phosphorus existed in organic type after digesting. According to the literature, the optimal N:P for corn was 7.5:1 (Lamsing et al., 2010), and we found the N:P result of PSS I was batter than the PSS II, which relatively closed to the recommended value. Therefore, the ratios of PM and RS should be lower than the using in PSS I in order to meet suitable N: P of fertilizer. Potassium is also an important nutrient for plants, due to potassium concentration was much higher than nitrogen as well as phosphorus, it shouldn't be a limiting

factor on fertilizer value for using the effluent of co-digestion with PM and RS.

4.8 Pathogens reduction during pseudo steady-state (PSS I and II)

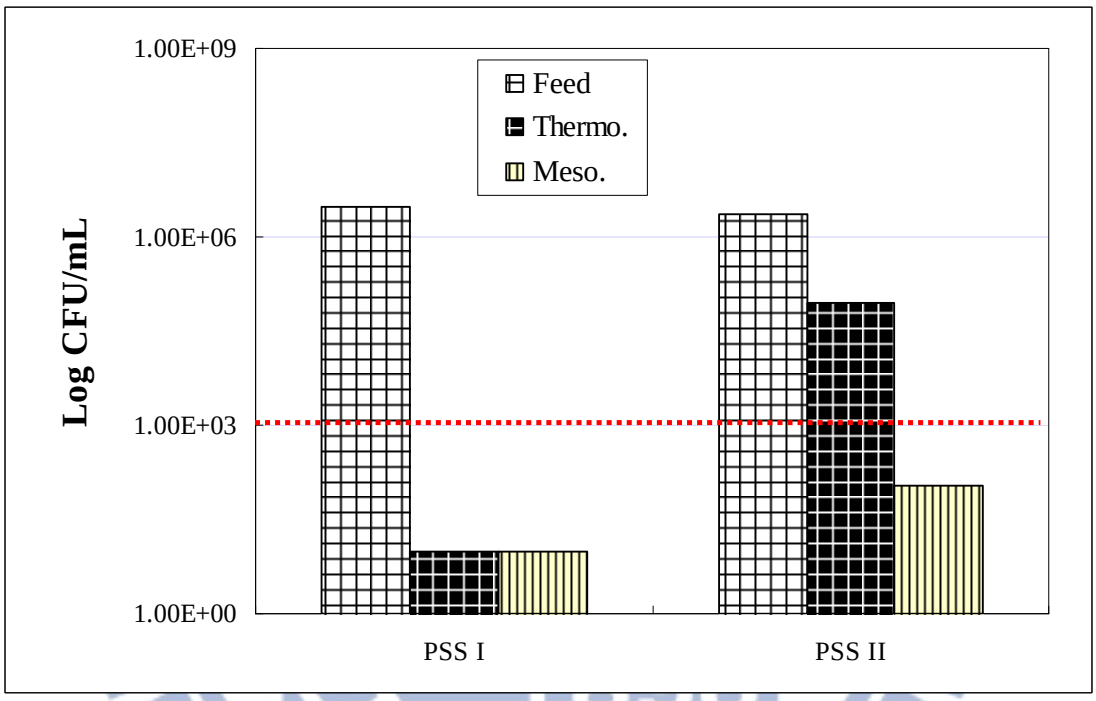


Fig. 4-4 The reduction of coliform group during PSS I and II

Fig. 4-4 is the result that coliform group were eliminated by TPAD system in PSS I and II. Overall, the TPAD system could effectively achieve both of Class A biosolids regulation and Taiwan's fertilizer standard for exterminating pathogens, which should be under the concentration: 1000 colony-forming unit (CFU)/mL (the dotted line in Fig. 4-4). The reduction result of coliform group in thermophilic stage of PSS II exceeded the standard due to equipment problems, although the final effluent was still below at the standard concentration due to diluted by a longer mesophilic HRT. Even if the problem was resolved, the coliform group

concentration of the effluent was still unable to down to a safe range. Moreover, it might be unable to meet the standard if operating at higher loading rates than the current one.

It should be noted that the reduction of pathogens in Class A biosolids regulation is based on fecal coliform removal and has to be under 1000 most probable number (MPN)/ g TS, besides, the MPN units is based on the multi-tube fermentation method rather than the filter membrane method, which uses the CFU unit. Gronewold and Wolpert (2008) created a model to investigate the relationship between MPN and CFU. And their conclusion indicated that MPN and CFU intra-sample variability didn't stem from human error or laboratory procedure variability, but was instead a simple consequence of probabilistic basis for calculating the MPN. So the accuracy in assessment of the microorganisms' concentration using the filter membrane method should be enough to trust when compared to multi-tube fermentation method.

4.9 Heavy metals during pseudo steady-state (PSS I and II)

Because the feed sources were agriculture and pig manure wastes, the heavy metals concentration shouldn't be high up to harmful levels. However Tab. 4-6 showed that concentrations of Cu and Zn were larger than other metals, these would be resulted from PM. Zn and Cu are important for growth of pig and due to their poor bioavailability, Zn and Cu usually are added at large levels exceeding physiological requirements. (Marcato et al., 2008). In addition, Cr concentration was higher in PSS I but decreased in PSS II; Ni concentrations both in PSS I and II were exceeded the Taiwan standard concentrations.

The specific control concentrations of heavy metals of liquid organic matter

fertilizers in Taiwan are: arsenic (As) shouldn't exceed 10.0 mg/kg; cadmium (Cd) shouldn't exceed 0.60 mg/kg; chromium (Cr) shouldn't exceed 30.0 mg/kg; copper (Cu) shouldn't exceed 20.0 mg/kg; mercury (Mg) shouldn't exceed 0.20 mg/kg; nickel (Ni) shouldn't exceed 10.0 mg/kg; lead (Pb) shouldn't exceed 30.0 mg/kg; and zinc (Zn) shouldn't exceed 160 mg/kg (台灣農糧署). Although, the concentrations of As and Mg didn't detect, these metals shouldn't be unlikely to harmful levels. The most likely causing the problem of heavy metals would be Cu and Zn. From the results, the heavy metals concentration of TPAD effluent didn't meet Taiwan fertilizer specifications either in PSS I or in PSS II, these results indicated the effluent from co-digestion of PM and RS if using as a biofertilizer should pay attention to the ratio of PM.

Tab. 4-6 Heavy metals concentrations of TPAD effluent during PSS I and II

	PSS I	PSS II
Cd (mg/kg DM [a])	ND [b]	0.2±0.0
Cr (mg/kg DM)	49.5±4.52	16.60±9.49
Cu (mg/kg DM)	99.9±10.06	79.47±5.33
Ni (mg/kg DM)	17.67±0.12	15.53±0.96
Pb (mg/kg DM)	13.37±6.99	2.73±0.19
Zn (mg/kg DM)	870.0±22.55	669.3±39.74

[a] Dry matter

[b] Not detected

Chapter 5 Conclusions and suggestions

Co-digestion of pig manure (PM) and rice straw (RS) operated at total 14 d HRT (T/M=4/10 d) had some consequences in this study. From the results, the feed concentration at 20 g VS/L and the ratio of PM and RS at 80:20 (PM:RS) were the better than the same concentration but ratio at 90:10 (PM:RS). TPAD system in this study faced a huge challenge due to the problems of equipments and operation, generally it still could achieve 40 CFR Part 503 regulations, which stipulate that VS removal should be more than 38%, the concentration of faecal coliform should be less than 1000 MPN/g TS or the concentration of *Salmonella* spp. should be less than 3 MPN/4g TS.

Compared to traditional single mesophilic AD operation treating single waste, TPAD system which applies the strategy of co-digestion two or more different sources wastes can not only obviously upgrade the whole system loading capacity but effectively eliminate pathogens if effluent using as a biofertilizer. The performances of AD system treating manure wastes have been limited by ammonia inhibition or treating carbohydrate-rich wastes have easily resulted in a large amount of VFAs as well as alkalinity rapidly declining. We can offset this shortcoming which usually occurs in a singe stage AD reactor through the concept of co-digestion various wastes.

Agriculture and livestock wastes after treated by TPAD system had a large potential to produce a biofertilizer or a soil conditioner in our results, and the thermophilic stage in TPAD system could effectively reduce pathogens, thus promoted the safety and sanitation of TPAD effluent, if it had other purposes. Although our results of heavy metals didn’t meet the Taiwan regulations for liquid

fertilizers especially in the Cu and Zn, besides, the concentrations of Cr and Ni were not higher than the former two metals but still exceeded the regulations due to an inappropriate ratio of PM and RS. These results indicated the ratios used in this study were not the optimal ratio of PM and RS, future researches should focus on ratios of co-digestion with various wastes.

Following suggestions contributed from this experiment provided some messages for researches of related topics in the future:

1. Rice straw should be conducted with physical treatment before digesting to avoid the obstruction possibility.
2. The optimal ratio of pig manure and rice straw should less than 80:20 (PM:RS) because the concentration of heavy metals still exceeded the fertilizer standards in this ratio.
3. We should pay attention to the operation of thermophilic reactor because operation of thermophilic microorganisms is quite difficult, and operation changes would cause fluctuations in thermophilic performances thereby extending the operation time.
4. Due to the substrate containing high solids concentration, it might result in obstruction in pumps or pipes or cause operational difficulties when using laboratory-grade equipments.
5. Although TPAD system can significantly eliminate pathogens, improper operation or equipment failure would appear the risk of pathogens concentration. The pathogens reduction still can’t effectively improve even though we extend the operation time.

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