Enhanced Dry Anaerobic Digestion of Swine Manure and Changes of Its Nutrients Bioavailability

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Abstract

Annually, large quantities of swine manure (SM) rich in organics, N and P contents are generated worldwide, which pose disposal problems and cause environmental issues like greenhouse gas emission and eutrophication in water. To minimize the harmful environmental impacts and to realize a sustainable agriculture, it is necessary to treat and stabilize these manure wastes and to fully recycle N and P nutrients. Dry anaerobic digestion (AD) is a promising method for bioenergy production and nutrients reclamation from the organic manure. Nevertheless, some limitations are ineluctable during this process regarding ammonia inhibition, volatile fatty acids (VFAs) buildup and restrictive hydrolysis of the fibrous materials in SM, which often result in long retention time and low overall recovery efficiency of the energy potential in SM. Therefore, this study aimed to develop proper and efficient pretreatment strategies to improve SM digestibility and to control ammonia inhibition and VFAs buildup during the dry AD. Special attention was also paid to N and P availability throughout the treatment.

In this study, two very different pretreatment procedures were adopted. First, short-term dry AD was conducted to promote SM hydrolysis and accumulation of ammonia, then ammonia recovery by air stripping and subsequent VFAs separation by water extraction were performed in order to minimize their inhibition during the dry AD. Second, low-temperature hydrothermal (HT) pretreatment was applied to accelerate manure hydrolysis and to prevent high ammonia load to the subsequent AD process. Major results are summarized below.

Dry AD of the tested SM at 55 °C, 20% TS and unadjusted initial pH (8.6) for 8 days produced relatively high concentrations of ammonia-N (20.0 mg/g-VS) and total VFAs (94.4 mg-COD/g-VS) with high potentially bioavailable P (10.6 mg/g-TS) remained in the digestate, which was considered optimal in this study. In addition, high ammonia removal efficiencies of 96.2% were achieved through 3 hours' dry stripping (operated at TS ~ 20%, 55 °C and initial pH 11.0), while the total VFAs concentration in the digestate remained favorably unchanged. Experimental data from the ammonia stripping processes well fitted to the pseudo first-order kinetic model (R^2 = 0.9916-0.9997). Compared with traditional air stripping handled in liquid form, ammonia stripping at high solids content has the advantageous of higher volumetric total ammonia nitrogen (TAN) removal rate

thus much smaller reactor volume, less energy/chemicals consumption and no foaming problems. After 8 days' dry AD and 3 hours' dry ammonia stripping, the separated liquid containing VFAs and the recovered ammonia were both marketable products, and the solid residues with averagely higher C/N ratios of 25.7 than those of raw SM (18.0) meanwhile maintaining a relatively high bioavailable P content of 8.1 mg/g-TS can serve as better feedstock for CH_4 fermentation.

HT pretreatment at 110 and 130 °C for holding 30 min did not significantly decrease the total solids content in raw SM, while an increase in soluble organic carbon (SOC) by 13.36% and 25.64% was obtained, respectively. Analysis of C mass balance revealed that carbohydrates, protein and VFAs were the major contributors to SOC (about 91%-92%) in the pretreated SM. Dry AD of the HT pretreated SM at 110 and 130 °C was performed successfully without alkali addition for pH adjustment. The pretreatment increased the overall VS destruction by 14.18%-18.98% and the final CH_4 production by 13.89%-33.70%. Energy balance analysis indicated a positive energy gain by adopting HT pretreatment at 130 °C. Except for the improvement in bioenergy yield, low-temperature HT pretreatment at 110-130 °C also promoted organic-N mineralization during the dry AD, which greatly increased the fraction of N that immediately available for plants in the digestate. After 70 days' dry AD, a high TAN to total nitrogen (TN) ratio of 70.67% was observed in the reactor loaded with SM that pretreated at 130 °C (R-130), in sharp contrast to that of 37.84% in the reactor treating raw SM (R-RSM). Low-temperature HT pretreatment apparently did not affect the overall P availability in SM. The existing forms of P in the digestate were mostly determined by the total multivalent metal ions (Ca^{2+}, Mg^{2+}, Fe^{2+}, Al^{3+}, etc.) content in the starting SM. And 23.21% of the P content in R-130 was potentially bioavailable after dry AD.

Both two proposed pretreatment procedures can serve as effective and promising alternatives to improve dry AD of the animal manure. Results from this work are expected to provide new concepts and useful information for the integral and comprehensive utilization of manure wastes in practice.

Key words: Swine manure; Dry anaerobic digestion; Ammonia; Phosphorus bioavailability; Volatile fatty acids; Hydrothermal technology

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Acronyms and abbreviations

AP:	Apatite phosphorus
COD:	Chemical oxygen demand
FAN:	Free ammonia nitrogen
HT:	Hydrothermal
IP:	Inorganic phosphorus
N:	Nitrogen
NAIP:	Non-apatite inorganic phosphorus
OP:	Organic phosphorus
Ortho-P:	Orthophosphate
P:	Phosphorus
RSM:	Raw swine manure
SM:	Swine manure
SMT:	Standards, Measurements and Testing
SOC:	Soluble organic carbon
SRT:	Solid retention time
TA:	Total alkalinity
TAN:	Total ammonia nitrogen
TKN:	Total Kjeldahl nitrogen
TN:	Total nitrogen
TON:	Total organic nitrogen
TP:	Total phosphorus
T(V)S:	Total (volatile) solids
TVFAs:	Total volatile fatty acids
VFAs:	Volatile fatty acids
WAS	Waste activated sludge

Chapter 1 Introduction

The world population has experienced expanding growth in the last century. Which directly resulted in a dramatic increase in energy consumption and food demand (crops, animal products, fruits, etc.). And the world is now facing many challenges, three of the most detrimental ones include the production of large quantity of organic wastes (e.g. animal manure) annually, energy related issues and alteration and disturbance of the global N and P cycles due to the use of chemical fertilizers in agriculture.

1.1. Manure wastes production and environmental issues

Intensive livestock industry annually produces staggering amounts of animal manure worldwide. Generally, manure wastes are generated in two forms, i.e. liquid manure and solid manure. According to a report published by MEP China (2014), the livestock husbandry in China annually releases 10.7 million tons of chemical oxygen demand (COD) and 0.6 million tons of ammonia-N. In Japan, the annual generation of livestock manure is about 83.0 million tons, of which 27% is swine manure (MAFF, 2015). In the US, the annual production of dry animal manure is estimated to be 35 million tons (Cantrell et al., 2008). And more than 1500 million tonnes of fresh manure is generated each year in the EU-27 (Holm-Nielsen et al., 2009). These large amounts of manure wastes represent a huge burden to the environment due to its high potential for pollution. In this sense, the related environmental issues mainly include surface and aquifer water pollution, air contamination, odors and greenhouse emission.

Inappropriate discharge of liquid manure to waterways often leads to serious eutrophication in lakes or rivers, characterized by high concentrations of nutrients (N and P) in the water that support abnormal growth of algae and aquatic plants like water hyacinths. As a result, oxygen levels in the waterbody decreased significantly, which poses a threat to the survival of other living organisms in the aquatic system. Moreover, the manure wastes contain a diverse range of pathogens including bacteria, viruses, parasites, yeast and fungi, and that represents a risk of infection to human or animals when it enters the drinking water resources. Studies have confirmed that some of the pathogens can survive for a long period of time and migrate significant distances through

soil horizontally or vertically (Abu-Ashour et al., 1994). Besides the potential for water pollution, poor handled animal manure could also cause air contamination. When the livestock manure undergoes natural biodegradation, it releases mainly carbon dioxide, methane, ammonia and nitrous oxides, which are among the major contributors to air pollution and global warming (UN FAO, 2006). It is reckoned that the worldwide livestock manure contributes about 300 million tons of atmospheric carbon dioxide and 18 million tons of methane annually (UN FAO, 2006). Meanwhile, the emission of gaseous ammonia (2 million tons per year) through mineralization of organically bound nitrogen in livestock manure has been a serious concern due to its negative impacts on the environment such as acid rain and eutrophication. For these reasons, proper and efficient methods are required for the treatment and management of manure wastes.

1.2. Increasing energy demand and global warming

The rapid increase in world energy consumption has raised public concerns over its supply problems and the negative environmental impacts such as air pollution, ozone depletion, and above all, global warming. The world had witnessed a striking increase in primary energy consumption by 49% from the year 1984 to 2004, and the growing trend is still continuing (Pérez-Lombard et al., 2008). According to the data released by US Energy Information Administration (US EIA, 2013), the global energy consumption will increase by 56% between 2010 and 2040, from 524 to 820 quadrillion British thermal units. Most of this growth will be contributed by the emerging countries, where the demand is fueled by the rapid growing economy.

Traditional fossil fuels (petroleum, coal and natural gas) are the major forms of energy in the global market currently, and will still be responsible for 80% of the energy use by 2040 (US EIA, 2013). As a result, the stocks of fossil based energy resources are depleting at a dangerously rapid speed. Moreover, the substantial amount of greenhouse gases emitted from fossil fuels burning has greatly altered the atmospheric environment and raised the mean temperature of earth surface by about 0.85 °C over the period of 1880-2012 (ICPP, 2014). To guarantee enough energy supply and to mitigate global warming, development of renewable energy is of priority.

1.3. Accelerated global N and P cycles

Both N and P are key elements for crops to grow. Traditional farming practice relies heavily on the application of animal manure to improve soil fertility. But in recent decades, this practice is handicapped by the availability of low cost chemical fertilizers. Each year, approximately 1% of the world's energy supply is consumed in order to generate the hydrogen gas (H_2) as well as the extreme conditions necessary for the synthesis of ammonia (Smith, 2002). And about 60% of the P consumed in farming derives from P rocks (Cooper et al., 2011).

The production and application of large amounts of synthesized fertilizers have profound influences on the environment. On the one hand, N and P nutrients in the animal manure are often not used to their potentials, which pose a burden to the environment. On the other hand, the large amounts of ammonia synthesized through Haber-Bosch process as well as the excessive mining of P rocks significantly increase the net input of reactive N and P components into the environment. This huge imbalance has led to accelerated global N and P cycles.

To cope with the above challenges, there is a clear demand for the recovery of energy and nutrient elements from manure wastes so that the environmental pollutions related with animal manure discharge and fossil fuels burning could be alleviated and the accelerated global N ad P cycles could be halted.

1.4. Current methods for manure wastes disposal

1.4.1. Composting

Composting is a prevalent process for manure management. It is the biological decomposition process, which takes place under conditions that allow the development of thermophilic temperature as a result of released heat, with the formation a final product that free of pathogens, parasites and weed seeds, and can be used to improve soil quality and fertility (Bernal et al., 2009). Composting is a simple process, which simply requires making a heap of organic wastes and waiting for them to decompose after a period of time. Modern composting process is often assisted by shredding the substrates, adding water and regularly turning the materials. The process has been used for centuries to stabilize the animal manure prior to their transportation and application. But frequently

the wastes are heated up with little regard to control of the reaction conditions like pH, temperature, oxygen level, greenhouse gas emission and nutrient loss. Tiquia et al. (2002) reported that during turned composting of the mixture of swine manure and cornstalk, 60%-68% of the organic-C content was emitted as CO_2, and 37%-59% of the total nitrogen (TN) and 23%-42% of the total phosphorus (TP) were lost.

1.4.2. Thermal drying

Thermal drying is a commercially available process for the dewatered manure. The process is known to facilitate the sanitation, transportation and storage of manure solids. And it has been successfully implemented for quick stabilization of the manure wastes at full-scale (MAFF, 2011). Nevertheless, thermal drying alters the nutrients content in animal manure, leading to a decreased agronomic value. Especially, the inorganic N content (mainly ammonia) is susceptible to loss via volatilization, which results in air pollution and a decrease in N fertilizer value.

1.4.3. Thermochemical process

Compared with traditional application of the animal manure as organic fertilizers, its utilization as feedstock for energy production could be a more sustainable choice. On this account, thermochemical processes like pyrolysis and combustion have been developed for direct conversion of manure wastes into energy.

Pyrolysis is the thermochemical decomposition of the organic wastes at elevated temperatures in the absence of oxygen for the production of gaseous fuels, liquid fuels and bio-char. Not only pyrolysis can be used as a single process for bio-energy production, but also it can serve as a primary step for combustion. By proper control of the reaction conditions (e.g. temperature, heating rate, holding time, etc.), pyrolysis can be used purposely and flexibly for the production of specific products. For example, a slow heating rate and long holding time favor the generation of bio-char (Xu and Chen, 2013). The manure-based bio-char obtained is a promising soil conditioner due to its rich nutrients content and high cation exchange capacity.

Combustion is the complete oxidation of organic wastes by oxygen at high temperatures to generate energy in the form of heat. And normally, the heat generated can

make the combustion self-sustaining. Advantages of the combustion process mainly include great waste volume decrease, complete degradation of VS and release of the energy potential in biomass. The ash that rich in nutrient elements (e.g. K, P, etc.) could be utilized as fertilizers when the content of heavy metals are less than the regulations.

Both pyrolysis and combustion are effective processes to realize waste-to-energy conversion, but their applications for animal manure treatment are still facing a lot of challenges. First of all, the capital investment cost is high for reactor construction. For pyrolysis, the energy input required by reactors treating livestock manure is sometimes higher than that produced (Fernandez-Lopez et al., 2015). Regarding the combustion process, the release of NO_x and SO_x is high due to the high N and S contents in manure wastes (Fernandez-Lopez et al., 2015).

1.4.4. Use as animal feed

Poultry waste such as litter or chicken manure has been used as feed ingredient to the ruminants for years. This waste contains large amount of minerals, proteins and fibers, and their application for animal feed is well documented and practiced in many countries and areas. However, potential rick of this process are the spread of pathogens and antibiotic-resistant bacteria from one animal to another.

1.5. Dry anaerobic digestion technology and enhancement measures

Anaerobic digestion (AD) has been recognized as a promising practice for animal manure stabilization due to its potentials for bioenergy production through organic matters decomposition, reduction in greenhouse gas emission, recycling of nutrients (N, P) and deactivation of pathogens (Abbasi et al., 2012; Massé et al., 2011). Traditionally, AD is performed in wet state with a total solids (TS) content < 15% (always ≤ 10% TS), and a large volume of water is required to achieve such a low TS condition. Despite its efficacy for biogas production, wet AD has some shortcomings such as large reactor volume, high construction cost, and discharge of large volume of digestate with high contents of organics and nutrients (mainly N and P), posing a serious threat to the surrounding water bodies. On the other hand, although this digestate can be re-utilized and applied on farmlands, a large proportion of N in the liquid digestate occurs as

ammonia-N which can easily lose and release during storage and land spreading, leading to insufficient nutrients utilization and air pollution. What's more, ammonia-N can be further nitrified in soil environment into nitrate, a highly movable form through the soil matrix to the aquifer, contributing to groundwater contamination (Alburquerque et al., 2012).

To optimize the economic efficiency for AD of livestock manure and to avoid the production of large quantities of liquid digestate, dry AD (TS ≥ 20%) can be adopted for the treatment of solid manure fraction obtained through solid-liquid separation, meanwhile the liquid fraction can be treated separately. Operation of AD in dry conditions contributes to smaller biogas facilities, easy handling of the digestate and minimal loss of nutrients (Karthikeyan and Visvanathan, 2013). Rico et al. (2015) reported a high methane (CH_4) yield of 265 ml/g-VS from thermophilic dry AD of dairy manure using percolate recirculation technology. High rate psychrophilic (20 ℃) dry AD of dairy manure at 35% TS has been proven to be possible when sufficient quantity of well acclimatized inocula was used (Saady and Massé, 2015). Chen et al. (2015) further justified the technical and economic feasibility of a continuous plug-flow dry AD reactor operated at ambient temperature for SM treatment.

Although being advantageous in size and costs of required facilities, some limitations are ineluctable during dry AD of livestock manure regarding restrictive hydrolysis of the fibrous materials in animal manure and thus subsequently long retention time and low overall anaerobic degradation efficiency of the manure volatile solids (VS, < 30%) (Menardo et al., 2011). Therefore, many studies have focused on increasing digestion rate and efficiency by promoting hydrolysis, which has been recognized as the rate-limiting step of AD.

1.6. Pretreatment strategies to accelerate organic manure hydrolysis

Pretreatment of the raw manure can reduce substrate viscosity and accelerate the disintegration of organic solids, thus increase their accessibility to the fermentative bacteria, which is expected to facilitate CH_4 production. Strategies that can promote organic matters hydrolysis mainly include controlled fermentation, hydrothermal (HT) technology, chemical, ultrasonic, extrusion and microwave process (Carlsson et al., 2012;

Carrere et al., 2016; Yuan et al., 2006; Zhang et al., 2010). Among them, controlled fermentation and HT technology are two effective and competitive options.

Controlled fermentation has the advantages of easy operation, mild reaction conditions and thus low requirement for equipment. By proper control of experimental conditions (temperature, pH, etc.), AD of swine manure can be restricted to hydrolysis and acidogenesis phases which can be purposefully used for organic solids solubilization and accumulation of fermentable products like soluble proteins, carbohydrates and volatile fatty acids (VFAs). Zhang et al. (2013) observed efficient hydrolysis of the WAS during controlled fermentation at alkaline conditions and a significant improvement in the final CH_4 yield. Yuan et al., (2006) also reported a high accumulation of total volatile fatty acids (TVFAs) at pH 10.0 after the waste activated sludge (WAS) being anaerobically fermented at ambient temperature for 8 days. The merits of HT pretreatment include short reaction period, no chemical addition, high processing capacity and sanitation effects. According to Huang et al., (in press), processing the chicken manure at 180 °C for 30 min resulted in 208% increase in soluble organic carbon (SOC) and improvement in the final CH_4 yield by 24%. High solubilization efficiency of 53.4% was observed with waste activated sludge (WAS) after HT treatment at 160 °C for 180 min (Xue et al., 2015).

Although controlled fermentation and HT process have both been successfully implemented at full-scale for improving biogas production from various organic wastes like animal byproducts, food wastes, sewage sludge and lignocellulosic materials (Carrere et al., 2016), little process data is available for their pilot- or full-scale application treating animal manure, especially that operated at high TS. One obstacle is the high N content in animal manure. Despite the efficacy of controlled fermentation and HT pretreatment for accelerating organic solids hydrolysis, the ammonia released concomitantly with organic manure disintegration is a problem of concern due to its toxicity to the methanogens during the AD process. Generally, free (unionized) ammonia is considered to be more toxic than ionized ammonia due to its high permeability through cell membrane (Rajagopal et al., 2013). Ammonia affects the bacteria in three ways: (1) induction of proton imbalance and subsequent retardation in material transportation; (2) extra energy is required to maintain proton balance in the cells through K^+ channels; (3) inhibition of specific types of enzymatic reactions (Rajagopal et al., 2013). Elevating concentrations

of ammonia during AD of animal manure could result in VFAs buildups by blocking the pathway of aceticlastic methanogenesis. Although VFAs are the direct precursors of CH_4, high accumulation of VFAs (especially propionic acid) in an AD system might induce toxicity to the methanogens, further aggravating this situation (Wang et al., 2009). During dry AD of SM, noticeable inhibition to CH_4 production was observed when ammonia-N concentration exceeded 3000 mg/L (Chen et al., 2015). In a solid-phase AD system developed for dairy manure treatment, high concentration of accumulated VFAs also brought about a decrease in organics degradation rate and daily CH_4 yield (Rico et al., 2015). Therefore, to guarantee high CH_4 yield from dry AD of the processed manure, it is important to find proper and innovative methods to control ammonia inhibition and VFAs buildup.

In this study, two treatment procedures were developed. First, after short-term dry AD of the manure wastes, ammonia recovery by air stripping and subsequent VFAs separation by water extraction were performed in order to mitigate their inhibition during dry AD. Both the recovered ammonia and the separated VFAs solution are valuable and marketable products. For example, ammonia can be applied as antimicrobial agents, alkaline cleaner, refrigerant gas, lifting gas, fuel, and most importantly as nitrogen (N) fertilizers for agriculture. And VFAs can serve as promising C resources to enhance nutrients removal from municipal wastewaters. In this sense, ammonia separation from the VFAs solutions can avoid extra N load to the wastewater treatment facilities. Second, low-temperature HT pretreatment was adopted in this work to prevent high ammonia load. Other benefits of the application of low-temperature HT pretreatment before dry AD include lower energy consumption and less extreme reaction conditions.

1.7. Research objectives and thesis structure

The objectives of this study were to investigate the feasibility of controlled fermentation and HT pretreatment for enhancing dry AD of the manure waste. Specifically, two pretreatment strategies were developed: (1) short-term dry AD followed by ammonia stripping and VFAs separation, and (2) low-temperature HT process. To achieve comprehensive utilization of the manure waste, special focus was also paid on

the retention of N and P bioavailability during the treatment procedure. Swine manure (SM) was selected as a typical example of animal manure in this study.

Main content of this thesis is composed of two parts. In Chapter 2, short-term dry AD (8 days) was employed for organic manure degradation and the production of ammonia and VFAs. The digestate was then stripped by air for ammonia recovery and extracted by water for the separation of VFAs. In Chapter 3, hydrolysis efficiency of the SM was investigated under two low HT temperature levels (110 and 130 °C). And CH_4 production from dry AD of the HT pretreated SM was also investigated and discussed. The thesis structure is displayed in Figure 1-1.
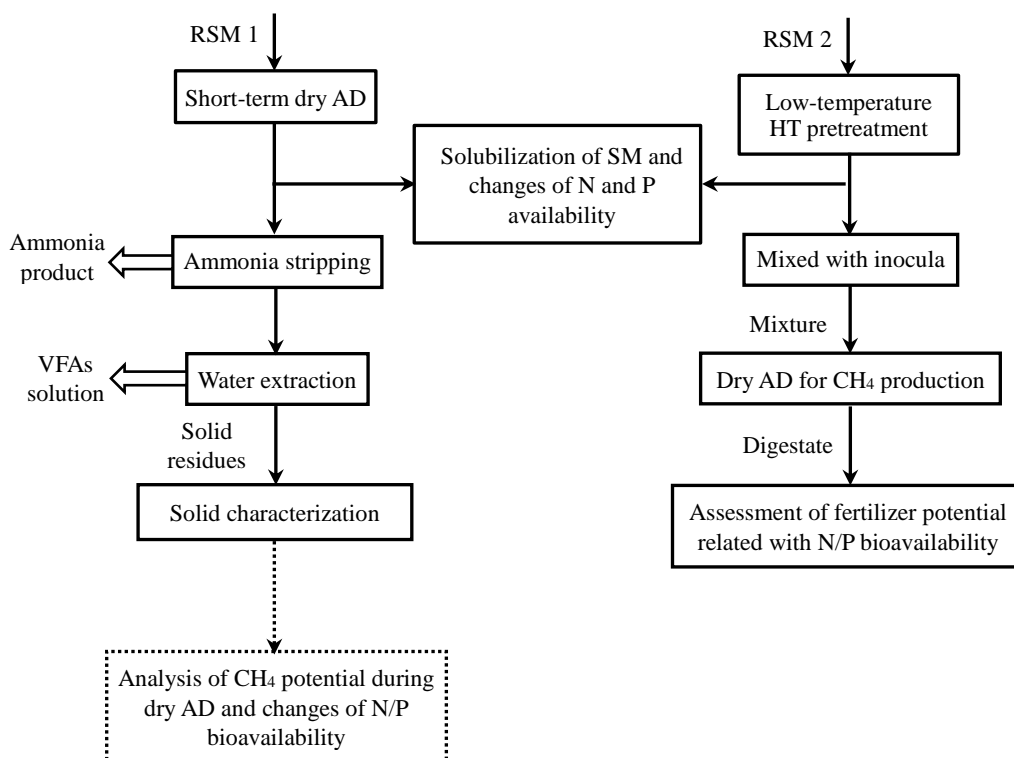
Figure 1-1. Experimental framework of this study. RSM-raw swine manure, AD-anaerobic digestion, HT-hydrothermal, VFAs-volatile fatty acids.

Chapter 2 Production of ammonia and volatile fatty acids from short-term dry anaerobic digestion of swine manure and their separation

2.1. Introduction

This chapter sought to maximize the utilization of the three major resources (C, N and P) in the manure, aiming at realizing the specific goals outlined as follows: (1) VFAs production from short-term dry AD of animal manure and its separation; (2) ammonia recovery from the digestate by stripping; and (3) preservation of high bioavailable P in the solid residue for further application (as solid fertilizer or feedstock for composting and/or CH_4 fermentation).

In order to obtain separated VFAs solutions and ammonia-N resource from SM, a two-step process involving dry AD followed by ammonia stripping was adopted as illustrated in Figure 2-1a. For ammonia stripping and final acquisition of the VFAs solutions, two strategies were tested in this study: (I) stripping of ammonia directly from the digestate in dry state followed by water dilution, mixing and solid-liquid separation (i.e. dry ammonia stripping); and (II) addition of water to the digestate, then solid-liquid separation, and finally ammonia stripping from the filtrate (i.e. wet ammonia stripping). Both the ammonia recovered from the digestate and the final liquid extract containing VFAs have market values. To the best of our knowledge, the feasibility of ammonia stripping from the digestate at TS $\geq$ 20% has not yet been documented.

In this chapter, VFAs and ammonia production efficiency was investigated under different dry AD conditions, and the fractionations of P before and after dry AD were revealed. Much attention was paid to the performance of ammonia stripping through the two proposed strategies, which was further compared in terms of technical and economic feasibility. Finally, the availability of VFAs, N and P resources in the liquid extract and solid residue obtained after the two-step treatment process were evaluated.
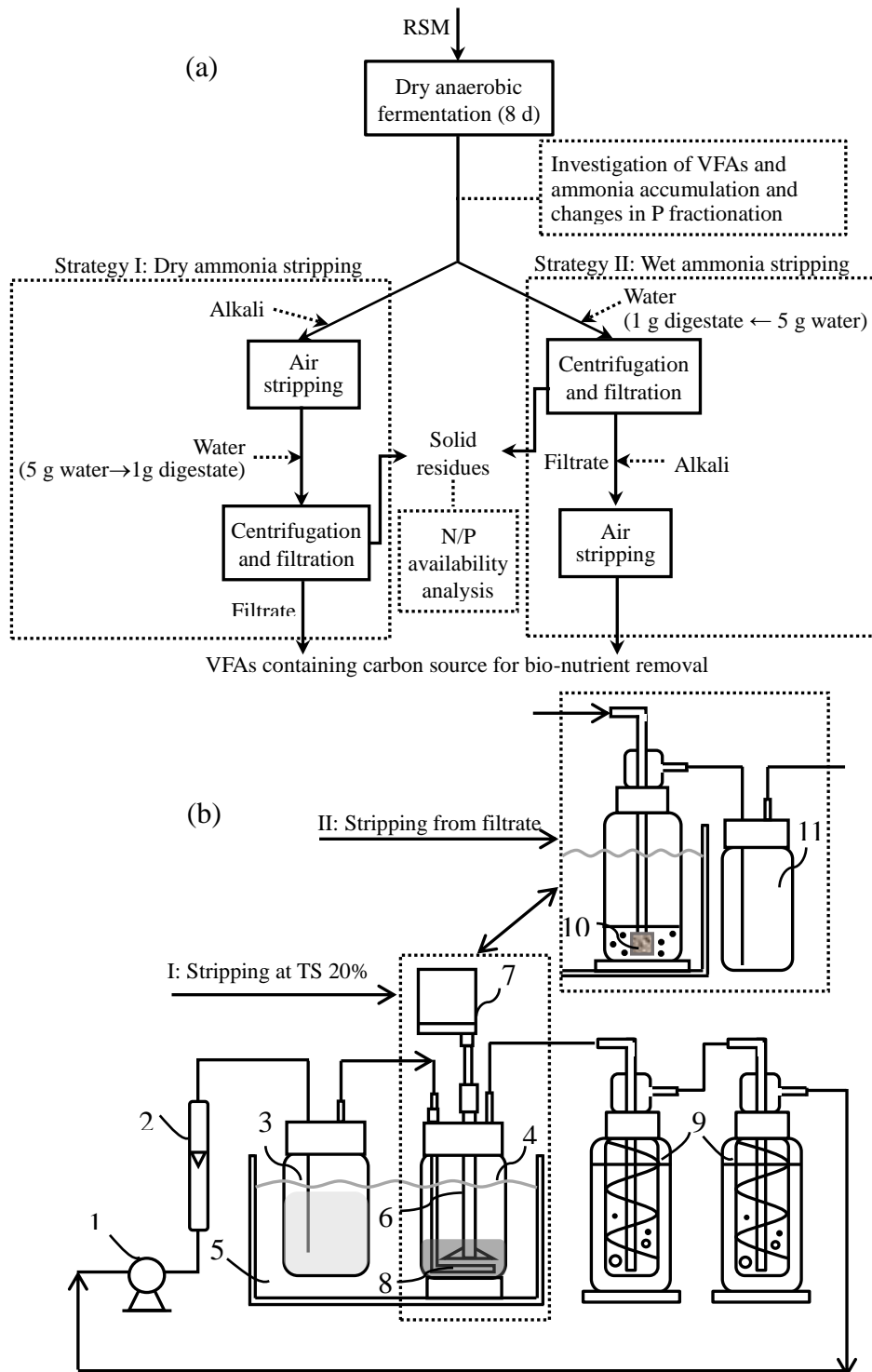
Figure 2-1. Schematics of (a) the procedure for VFAs separation from the fermented swine manure; and (b) the recirculating system for ammonia stripping and recovery. 1-gas pump, 2-gas flow meter, 3-vessel with water, 4-vessel with digestate, 5-temperature controlled thermostat, 6-propeller, 7-motor, 8-circular hollow tube with openings at the bottom, 9-acid absorption bottles, 10-porous ceramic head, 11-buffer bottle.

2.2. Materials and methods

2.2.1. Raw swine manure

Raw swine manure (RSM) was collected from a pig farm in Ibaraki, Japan. Chopped straw was used as bedding materials in the pig house. Solid RSM containing straw was sampled directly from the floor of pig house. The obtained RSM was mixed thoroughly and stored at 4 °C before experimental analyses, and its main characteristics are presented in Table 2-1 based on five tests in parallel.

Table 2-1. Characteristics of the raw swine manure used in the experiments.

Parameters	Raw swine manure (RSM)
Total solids (TS)	37.1 ($\pm$0.2) %
Volatile solids (VS, TS based)	77.6 ($\pm$0.2) %
Total ammonia nitrogen (TAN)	10.6 ($\pm$0.3) mg/g-VS
Total organic nitrogen (TON)	19.3 ($\pm$1.1) mg/g-VS
Organic phosphorus (OP)	3.8 ($\pm$0.3) mg/g-TS
Apatite phosphorus (AP)	7.0 ($\pm$0.5) mg/g-TS
Non-apatite inorganic phosphorus (NAIP)	7.3 ($\pm$0.5) mg/g-TS
Total volatile fatty acids (TVFAs)	39.1 ($\pm$1.1) mg-COD/g-VS
C/N	18.0 ($\pm$0.5)
pH	8.6 ($\pm$0.1)

The data were expressed as mean ($\pm$SD).

2.2.2. Short-term dry AD for VFAs and ammonia production

The SM was anaerobically incubated at controlled experimental conditions, i.e. temperature ranging from 25 °C to 55 °C, TS content from 20% to 35%, and initial pH from 7.0-12.0, respectively. The initial system pH was adjusted with 6 M HCl solution or solid $Ca(OH)_2$. In this section, $Ca(OH)_2$ was used due to its low cost, wide availability, and better performance for VFAs production than other alkalis like NaOH, KOH, and CaO according to my preliminary tests. For the dry AD trials, 12 identical cylindrical reactors (4.4 cm in diameter, 7 cm in height) with working volume of 100 ml were used. The manure was added with water and mixed thoroughly to achieve a designed TS content.

And 90 g of the mixture was loaded into each reactor. The reactors were then flushed with N_2 for 2 min, and sealed with silicone stoppers. Then the reactors were placed in temperature-controlled water bath and incubated at the designed temperature for 8 days, and three of them were sacrificed for determination of related parameters every other day.

To get sufficient amount of VFAs and ammonia enriched digestate for the stripping experiments (Figure 2-1b), an anaerobic reactor (18.8 cm in diameter, 14.5 cm in height) with a working volume of 4 L was operated for 8 days without mixing under the optimal conditions determined in the above trials. The digestate was then homogenized and stored at 4 °C before used for ammonia stripping experiments.

2.2.3. Ammonia stripping

Ammonia stripping from the solid digestate was conducted in an enclosed system as illustrated in Figure 2-1b. For dry ammonia stripping, 130 g digestate was loaded into a 500 ml glass vessel equipped with motor-driven propeller. As for wet ammonia stripping, 130 g liquid was introduced into a bubbling reactor with a working volume of 500 ml and a buffer tank was followed to prevent the foams from entering the acid solution bottle. Air was firstly pumped into a vessel containing water to pre-warm the gas and to compensate the moisture loss from the stripping reactor. It was then flushed into the digestate containing vessel through a circular tube with small openings (ϕ2) at the bottom or purged into the bubbling reactor and carried the volatile ammonia into the HCl absorption bottles (500 ml×2, 1.5 M) for entrapment. The gas was circulated among the vessels and acid solutions at a gas flow rate of 216 ml/min. Temperature and pH were tested respectively at two levels (35 °C, 55 °C and initial pH of 10.0 and 11.0) for a stripping duration of 3 hours. NaOH was applied for pH adjustment in the ammonia stripping experiments due to its efficacy and retention of P availability. Concentrations of ammonia and TVFAs in the solid digestate/liquid filtrate were detected every 30 min during the stripping process.

For solid-liquid separation, the mixture of water and digestate was centrifuged at 9000 rpm and ambient temperature for 20 min and then filtered by filter papers. Characteristics of the VFAs containing solution and the final solid residue obtained

through the two proposed strategies (Figure 2-1a) under their optimal stripping conditions were also tested.

2.2.4. Analytical method

TS content was measured by drying the manure sample at 105 °C till constant weight, and volatile solids (VS) content was determined by incinerating the dried manure at 600 °C for 3 hours. C/N ratio of the manure was measured using an organic element analyzer (Perkin-Elmer 2004 CHN, USA). The manure pH was measured with a semi-solid pH meter (Testo 206, Germany). TN and total Kjeldahl nitrogen (TKN) were determined by adding known amount of deionized water into 1 g solid manure and then analyzing the mixture in accordance with standard method (APHA, 2012). Total organic nitrogen (TON) was calculated as the difference between TKN and total ammonia nitrogen (TAN).

For analysis of soluble products, 4 g manure sample (wet weight) was diluted with 40 ml deionized water. The mixture was centrifuged at 9000 rpm and ambient temperature for 20 min and then filtered through a 0.45 µm microfiber filter. Measurements of TAN, ortho-P and soluble chemical oxygen demand (SCOD) were conducted in accordance with standard methods (APHA, 2012). The filtrate was acidified by 3% phosphoric acid solution to pH around 4.0 before VFAs analysis by a Shimadzu GC-14B/FID packed with Unisole F-200 30/60 column. The column and the injector temperatures were set at 150 °C and 180 °C, respectively. The pressure of N_2 carrier gas was maintained at 200 Kpa. In this chapter, the concentrations of VFAs were presented as equivalent COD values calculated from the theoretical formula of each VFA component.

Hydrolysis has long been recognized as the rate-limiting step for protein disintegration. To investigate the key enzyme activity related to protein hydrolysis and subsequent ammonia production, protease activity was measured on day 6 and at the end of each experiment according to the procedure described by Jang et al. (2014). One unit of protease activity (U) was defined as the amount of protease in the digestate capable of hydrolyzing casein to 1 µg tyrosine within 1 min at the respective cultivation temperature.

To reveal the dynamic change of P species in SM before and after dry AD, fractionation of P was conducted according to Standards, Measurements and Testing

(SMT) Programme extraction protocol (Medeiros et al., 2005; Ruban et al., 1999). Details of P fractionation are illustrated in Figure 2-2. P in the solid SM was classified into 2 categories: organic phosphorus (OP) and inorganic phosphorus (IP). Two main forms of IP were fractionated, i.e. bio-available non-apatite inorganic phosphorus (NAIP) loosely bound on exchange sites or associated with Al, Fe and Mn oxide, and Ca-bound apatite phosphorus (AP) which is not able to be utilized by most microorganisms and plants (Manning et al., 1984; Ruban et al., 1999). TP was the sum of OP and IP. The fractions of P that can be potentially released and utilized by microorganisms and plants were termed potentially bioavailable P (i.e. OP and NAIP).
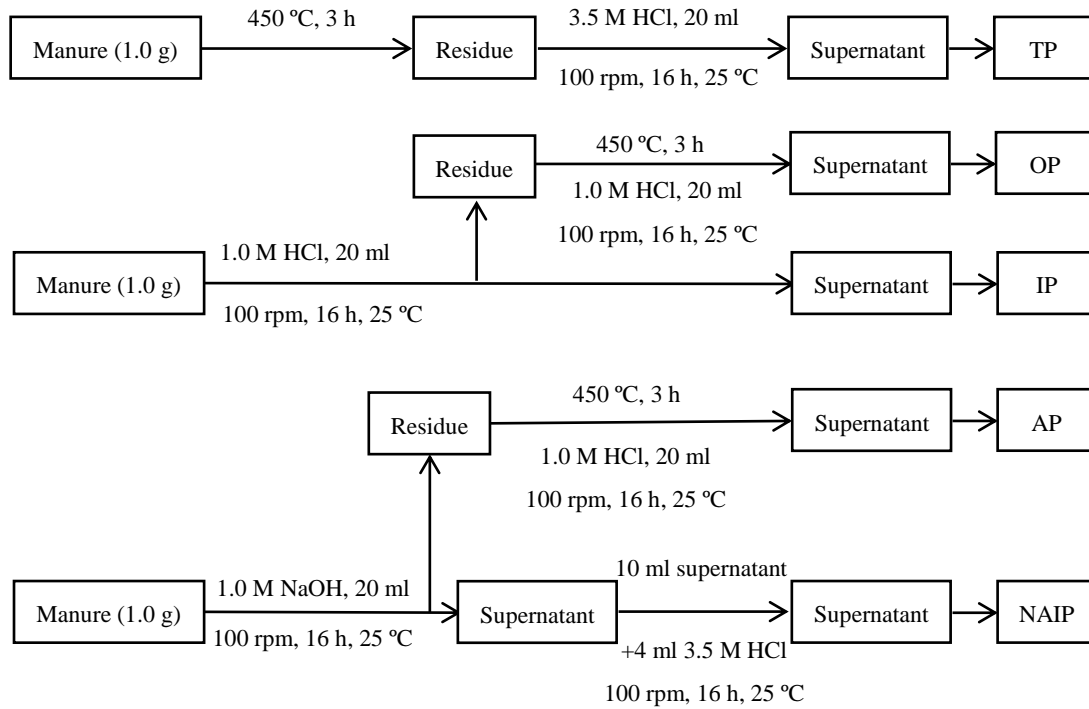
Figure 2-2. Schematic diagrams for P fractionation using SMT protocol (Medeiros et al., 2005; Ruban et al., 1999).

2.2.5. Calculations and kinetics

Concentration of free ammonia nitrogen (FAN) was calculated according to the following Eq. 2-1 (Hansen et al., 1998):

$$FAN = TAN \times \left(1 + \frac{10^{-pH}}{10^{-\left(0.09018 + \frac{2729.92}{T}\right)}}\right)^{-1} \qquad 2\text{-}1$$

where T is the temperature (K).

Ammonia-N yield refers to the amount of TAN produced during the fermentation and was defined by Eq. 2-2. Average organic-N conversion rate (mg/g⁻VS/d or mg/g-TON/d) and organic-N mass conversion ratio (%) were employed to assess the efficiencies of ammonia release under different fermentation conditions according to Eq. 2-3 and Eq. 2-4, in which the former was calculated based on initial VS or TON level of the SM used in the experiments, respectively.

Ammonia-N yield (mg/g-VS) = [Final TAN] − [Initial TAN] $\hspace{2cm}$ 2-2

Organic-N conversion rate (mg/g-VS/d) $= \dfrac{\text{[Final TAN]} - \text{[Initial TAN]}}{\text{SRT}}$ $\hspace{1.5cm}$ 2-3

Organic-N mass conversion ratio (%) $= \dfrac{\text{[Final TAN]} - \text{[Initial TAN]}}{\text{[Initial TON]}}$ $\hspace{1cm}$ 2-4

in which solid retention time (SRT) is 8 d in this chapter, and initial and final TAN concentrations are in mg/g-VS. The values of organic-N conversion rate in mg/g-TON/d were obtained by dividing those in mg/g-VS-d by the initial content of TON in the tested SM.

Pseudo first-order kinetic model expressed as Eq. 2-5 was applied for process analysis in order to disclose the mechanisms of ammonia stripping at dry or wet state.

$A_t = A_{eq} (1-e^{-kt})$ $\hspace{2cm}$ 2-5

where t (min) is the stripping duration, and k (min⁻¹) the TAN removal rate constant. A_{eq} and At (%) represent the TAN removal efficiency at equilibrium and time t, respectively.

In addition to TAN removal efficiency, effective TAN removal duration (τ_e, min) defined as the stripping duration for achieving 80% TAN removal was used to indicate the performance of the stripping process.

2.2.6. Statistics analysis

Each experiment (fermentation or ammonia stripping) was performed in triplicate. All analyses of solid and liquid samples were repeated twice and the results presented were mean values of six repetitions. For data analysis, one-way analysis of variance (ANOVA) was applied to compare the difference in ammonia production under different operation conditions by using SPSS 19.0 (IBM, US). Statistical difference was assumed significant at $p < 0.05$.
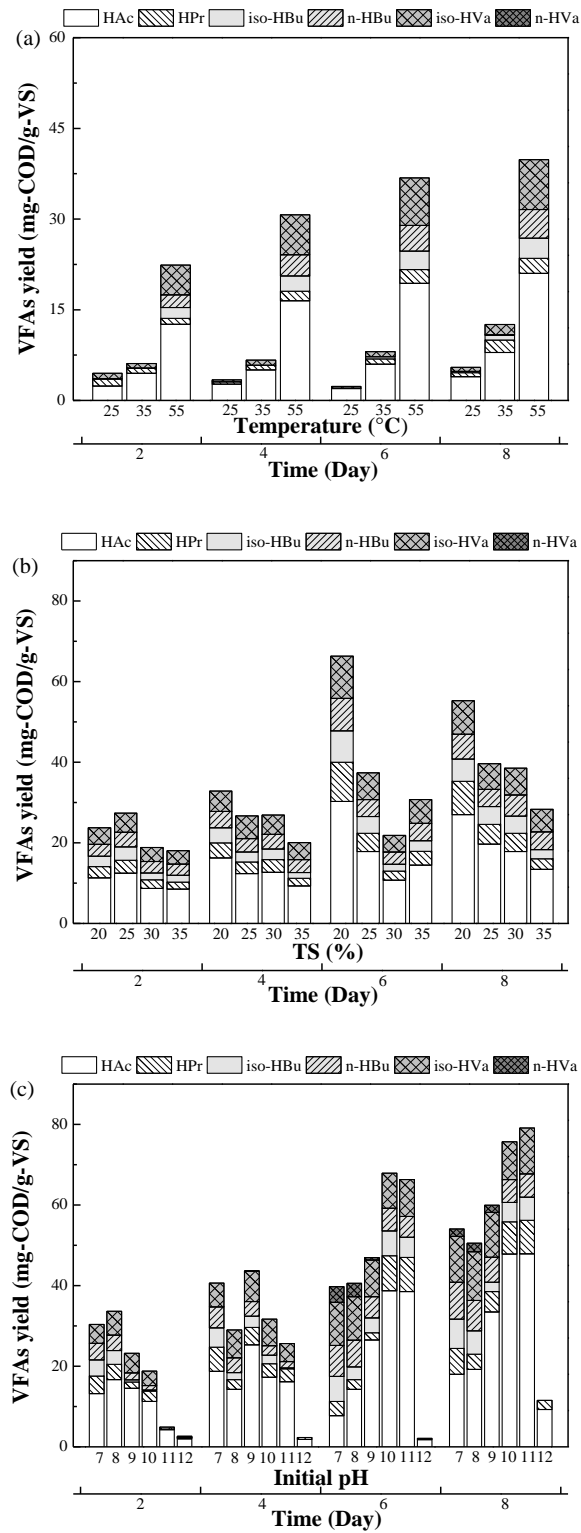
Figure 2-3. Effects of temperature (a, TS=25% without pH adjustment), TS content (b, 55 °C without pH adjustment) and initial pH (c, 55 °C and 20% TS) on dry AD for the production of VFAs. Acetic acid (HAc), propionic acid (HPr), iso-butyric acid (iso-HBu), n-butyric acid (n-HBu), iso-valeric acid (iso-HVa) and n-valeric acid (n-HVa).

2.3. Results and discussion

2.3.1. VFAs production from SM during 8 days' dry AD

The effect of temperature on net VFAs yield at 25% TS without initial pH adjustment (pH~8.6) is shown in Figure 2-3a. It is clear that an increase in temperature favored the production of VFAs. After 8 days' dry AD, a low TVFAs yield of 5.5 mg-COD/g-VS was obtained at 25 °C. In comparison, the final TVFAs yield was increased by 1.3 and 5.8 times at 35 °C and 55 °C, about 12.6 and 37.2 mg-COD/g-VS, respectively. The VFAs detectable during dry AD of SM were mainly short-chain fatty acids with 2-5 C atoms, including acetic, propionic, iso-butyric, n-butyric, iso-valeric and trace of n-valeric acids. Acetic acid (HAc) was the dominant VFAs product at all tested temperatures, accounting for 71.7%, 62.9% and 56.6% of the TVFAs produced after 8 days' dry AD of SM at 25 °C, 35 °C and 55 °C, respectively. Propionic acid (HPr) was the second most prevalent VFAs product at 25 °C and 35 °C, respectively accounting for 11.9% and 16.4% of the TVFAs generated at the end of fermentation, whereas iso-valeric acid (iso-HVa) was the second major VFAs at higher temperature of 55 °C, and its percentage in the produced TVFAs remained pretty stable between 22.2-24.2% ($p = 0.3962 > 0.05$) throughout the whole dry AD process at this thermophilic temperature. In view of VFAs production efficiency, 55 °C was considered to be the most favorable among the three tested temperatures.

In this section, dry AD of SM was performed at 55 °C and different TS contents (without initial pH adjustment), and the profiles of VFAs yield are shown in Figure 2-3b. At 20% TS, the TVFAs yield increased with fermentation time and reached a maximum of 66.4 mg-COD/g-VS on day 6. After that, obvious VFAs consumption was observed, leading to decreased final TVFAs yield to 55.3 mg-COD/g-VS at the end of fermentation. As for TS contents of 25%, 30% and 35%, the highest TVFAs yields were recorded as 39.6 mg-COD/g-VS on day 8, 38.5 mg-COD/g-VS on day 8, and 30.7 mg-COD/g-VS on day 6, respectively. During the dry AD of SM at 55 °C, HAc was the most abundant VFAs product at all tested TS levels, followed by iso-HVa.

It is noteworthy that under the same operational conditions (55 °C, TS 25%, and initial pH~8.6), the results of VFAs yield and its composition obtained in these experiments were slightly different from those presented in section 3.1.1. A similar phenomenon was also noticed for ammonia production and P fractionation, most probably

attributable to the complexity of dry AD process and heterogeneous nature of the SM investigated. Considering the efficiency of VFAs production, 20% TS was applied in the following tests.

The effect of different initial pH on VFAs production at 55 °C and 20% TS is demonstrated in Figure 2-3c. As shown, the highest yields of TVFAs were detected on day 8 at all tested initial pH levels, which followed a descending order as pH 11.0 (79.1 mg-COD/g-VS) > pH 10.0 (75.7 mg-COD/g-VS) > pH 9.0 (60.0 mg-COD/g-VS) > pH 7.0 (54.0 mg-COD/g-VS) > pH 8.0 (50.5 mg-COD/g-VS) > pH 12.0 (11.5 mg-COD/g-VS). Obviously, initial pHs of 11.0 and 10.0 were beneficial for VFAs accumulation. Despite a slightly higher TVFAs yield was obtained at initial pH 11.0 than that at pH 10.0, their difference was statistically insignificant ($p = 0.4589 > 0.05$). After 8 days' dry AD at 55 °C, 20% TS and initial pH 8.0-10.0, high volumetric TVFAs production rate of 979.7 to 1468.6 mg-COD/L/d was achieved in this study.

After 8 days' fermentation, the proportion of HAc yield to TVFAs yield was in the following order: pH 12.0 (80.4%) > pH 10.0 (63.2%) > pH 11.0 (60.5%) > pH 9.0 (55.8%) > pH 8.0 (38.0%) > pH 7.0 (33.3%). It is apparently that the proportion of HAc increased almost linearly with the increase of initial pH, suggesting that HAc production pathway is more dominant and robust than the production of other VFAs under alkaline dry AD conditions.

It is interesting to notice that, when operated at 55 °C, 20% TS and similar initial pHs of 8.0 (adjusted with HCl), 8.6 (unadjusted) and 9.0 (adjusted with $Ca(OH)_2$), the patterns of VFAs accumulation were quite different (Figs. 2b and 2c). At adjusted initial pHs 8.0 and 9.0, the TVFAs yield increased gradually during the dry AD process and reached a maximum on day 8 although impermanent stagnation seemed to occur during days 2-4 and days 4-6, respectively. In comparison, quicker TVFAs accumulation was observed at unadjusted initial pH 8.6 with higher maximum TVFAs yield on day 6. This observation was most probably brought about by the disturbance of chemical (HCl or $Ca(OH)_2$) addition on the microbial communities localized in SM, that is, some adaptation or acclimation was necessary for the fermentation bacteria to accommodate to the resultant microenvironment like elevated ionic strength or FAN.
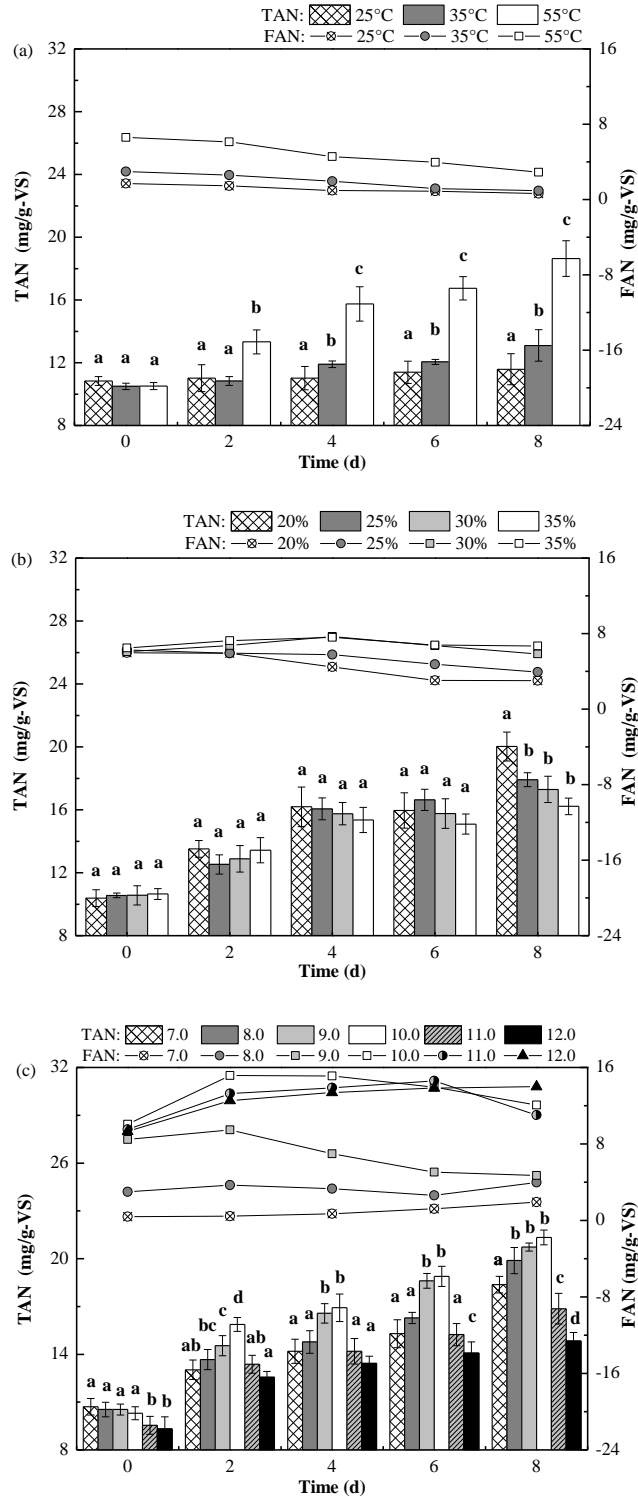
Figure 2-4. Effects of different temperatures (a, TS was fixed at 25% and initial pH was not adjusted), TS contents (b, temperature was fixed at 55 °C and no initial pH adjustment was done) and initial pHs (c, temperature was fixed at 55 °C and TS was fixed at 20%) on dry AD for ammonia production. Different letters (a to d) indicate significant difference of TAN ($p < 0.05$).

2.3.2. Ammonia production during dry AD of SM

In the batch experiments, anaerobic fermentation was conducted under different temperatures at 25% TS without pH adjustment (pH~8.6). As shown in Figure 2-4a, temperature played an important role in anaerobic ammonification with respect to ammonia production, organic-N mass conversion ratio or organic-N conversion rate ($p = 0.0124$-$0.0171 < 0.05$). Namely, higher temperature favors the release of TAN from SM. After 8 days' fermentation at 25 °C, only 3.9% of the original organic-N was converted into ammonia (Figure 2-4a), and the average organic-N conversion rate was calculated to be 0.1 mg/g-VS/d. Increase of the fermentation temperature by 40% (to 35 °C) and 120% (to 55 °C) resulted in increased organic-N mass conversion ratio by 2.5 times and 9.8 times, respectively. During the 8 days' dry AD, the mass ratio of TAN to TN at 55 °C and 25% TS increased gradually from initial 34.8% to 61.7%, with a final TAN concentration of 18.6 mg/g-VS being achieved.

Protein is the predominant organic matter responsible for ammonia release from dry SM in this study. During anaerobic fermentation, it should be initially hydrolyzed into polypeptides, followed by amino acids, and then converted into short-chain organic acids, ammonia and carbon dioxide (CO_2). Protease activity tests further confirmed the positive effect of temperature increase on ammonia fermentation (Figure 2-5a): After the fermentation, the highest protease activity of 1367.3 U/g-VS was detected at 55 °C, followed by 713.6 U/g-VS at 35 °C and 662.3 U/g-VS at 25 °C, respectively. An increase in temperature clearly accelerated the enzyme-mediated reaction of ammonia production. The system pH dropped gradually from around 8.6 to 8.0 at 25 °C, to 7.8 at 35 °C and to 7.7 at 55 °C, respectively (Figure 2-5a). These observations are to a certain degree inconsistent with the results from Abouelenien et al. (2009a) who pointed out that when varied from 35 °C to 65 °C, temperature had a statistically insignificant impact on TAN production from chicken manure after 8 days' dry AD. This could be attributed to the different characteristics of the manure used in the experiments, especially the different initial TKN and TAN concentrations. Another contributor to this difference may be the much higher initial TKN content in the tested chicken manure (Hansen et al., 1998) (Table 2-2) which might be toxic to ammonifying bacteria during ammonia fermentation,

resulting in no significant difference in ammonia production at different treatment temperatures.

In order to verify the negative effect of excess TAN on ammonia production, additional ammonia fermentation of SM was conducted at high initial TAN load of 23.7 mg/g-VS by adding ammonium chloride (TAN in the RSM was 10.6 mg/g-VS). Other parameters including temperature, TS and initial pH were kept identical with the above batch experiments. As expected, the organic-N mass conversion ratio was declined by 25.7%, 34.2% and 13.1% at 25 °C, 35 °C and 55 °C, respectively. Accordingly, lower protease activities were also detected at the end of the fermentation, about 447.7 U/g-VS at 25 °C, 684.8 U/g-VS at 35 °C and 1059.7 U/g-VS at 55 °C, suggesting the inhibiting effect of high TAN concentration on protein hydrolysis.

Ammonia is accumulated as a byproduct during the fermentation process. Although FAN concentration was always the highest in the reactors, about 3 mg/g-VS to 6 mg/g-VS, the highest ammonia production was achieved at 55 °C (Figure 2-5a). When lasting for about 4 days to 6 days, ammonia generation process was found to be temporarily retarded or ceased and then recovered to some extent probably brought about by the inhibition effect of ammonia to the bacteria, which could be ameliorated by other co-existing substances like VFAs produced or the adaptation of bacteria to the high level of ammonia. This phenomenon is partially evidenced by the results of Nakashimada et al. (2008) who observed an initial increase in TAN to around 5.8 mg/g-TS and subsequent flattening out after 4 days' AD of WAS under both thermophilic and mesophilic conditions. They further claimed that temperature mainly affected the kinetics of ammonia release instead of the final TAN concentration. Considering ammonia production efficiency and organic-N mass conversion ratio, 55 °C was selected as a preferable temperature for ammonia release from SM by dry AD in the following experiments.
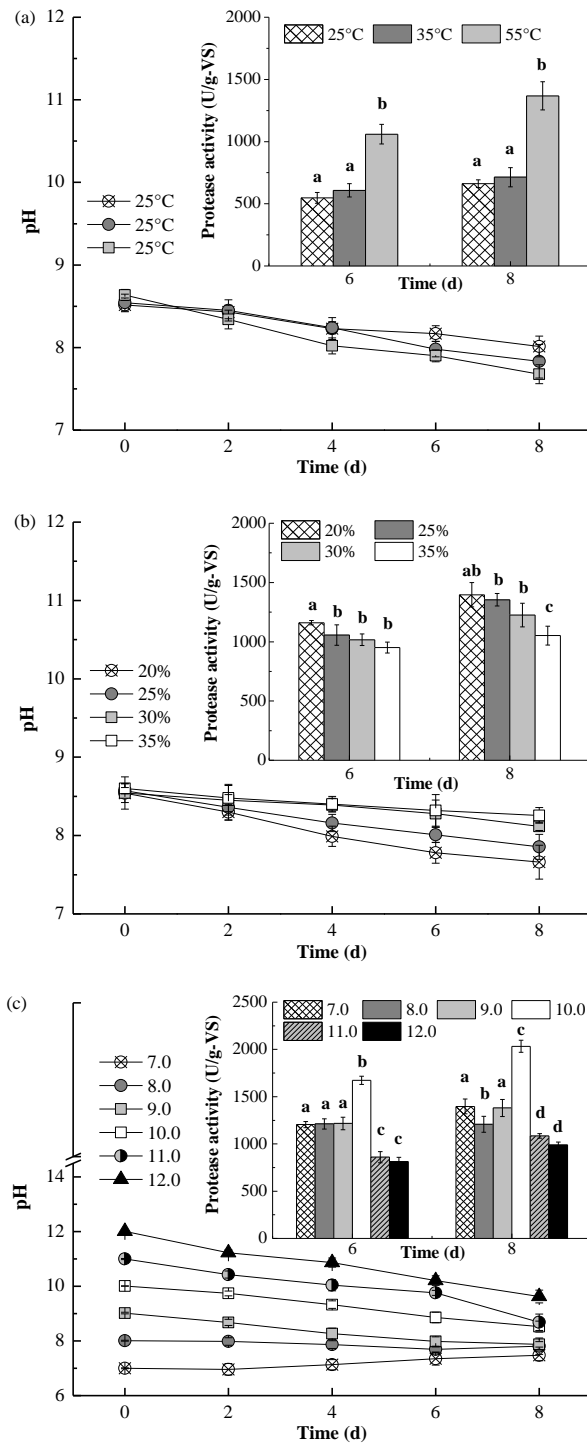
Figure 2-5. Variations of pH throughout the experiment and protease activity at day 6 and 8 under different dry ammonia fermentation conditions: (a) Effect of temperature (TS was fixed at 25% and pH was not adjusted); (b) Effect of TS content (temperature was fixed at 55 °C and no pH adjustment was done); (c) Effect of initial pH (fixed temperature at 55 °C and TS at 20%). Different letters (a to d) indicate significant difference of protease activity ($p < 0.05$).

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Figure 2-4b illustrates the variation of TAN and FAN at different TS contents during thermophilic fermentation (55 °C). In the end of the fermentation, the highest levels of ammonia-N yield, organic-N mass conversion ratio and average organic-N conversion rate were achieved at 20% TS, about 9.7 mg/g-VS, 50.1%, and 1.20 mg/g-VS/d, in comparison to those of 5.6 mg/g-VS, 29.0%, and 0.7 mg/g-VS/d at 35% TS, respectively. That is, when TS content was increased from 20% to 35%, both organic-N mass conversion ratio and average organic-N conversion rate were decreased by about 42%. This observation is possibly attributable to the nature of dry AD as a mass-transfer controlled process (Martin et al., 2003). After 8 days' fermentation, the TAN to TN mass ratio in the substrate increased from initial 34.4% to 66.3% at 20% TS. It is noticeable that under the same operation conditions (55°C, TS 25%, and pH~8.6), the results of TAN or FAN concentration presented in this section were slightly different from those in section 3.1.1, most probably due to the heterogeneity nature of the raw materials and dry AD process. Small difference was also discerned regarding protease activity, pH, gas production and kinetics as described below.

The system pH dropped gradually from around 8.6 to 7.7, 7.9, 8.1 and 8.3 at TS 20%, 25%, 30% and 35% on day 8, respectively (Figure 2-5b). An increase in TS content from 20% to 35% significantly increased the diffusive resistance of substrates, reducing the effective contact between enzymes/microorganisms and organic substrates. Furthermore, higher TS content may also contributed to higher localized concentrations of TAN, aggravating ammonia inhibition to the fermentative bacteria thus affecting the activity of extracellular protease activity. As seen from Figure 2-5b, the protease activity at the end of the fermentation decreased from 1396.7 U/g-VS at 20% TS to 1354.4, 1224.8, and 1052.1 U/g-VS at TS contents of 25%, 30% and 35%, respectively. Increase in TS content lowered the protease activity, resulting in decreased efficiency of ammonia production. It is noticeable that increase in TS from 20% to 25% resulted in significant decrease ($p < 0.05$) of the final TAN concentration (Figure 2-4b) and protease activity (Figure 2-5b). Whereas the TAN concentration and protease activity were statistically insignificant among TS 25% to 35% (Figure 2-4b and Figure 2-5b). In view of ammonia release efficiency, a TS content of 20% was considered to be optimal in the present study. Under the tested TS content conditions, ammonia production was also observed to retard to some extent around day 4 to day 6 and seemed to be recovered after this period (Figure 2-4b).

Table 2-2. Comparison of efficiencies of organic-N conversion to ammonia-N under different anaerobic fermentation conditions.

Manure	I/S[b]	Temperature (°C)	TS (%)	Initial pH	Operation mode	SRT (d)	Initial TKN (mg/g-VS)	Initial TON (mg/g-VS)	Initial TAN (mg/g-VS)	Average rate (mg/g-VS/d)	Average rate (mg/g-TON/d)	Ratio (%)	Reference
										Organic-N mass conversion[a]			
Swine	0	55	20	8.6*	Batch	8	29.8	19.3	10.6	1.2	62.2	50.1	This work
Swine	0	55	20	7.0	Batch	8	29.8	19.3	10.6	1.0	50.0	39.8	This work
Swine	0	55	35	8.6*	Batch	8	29.8	19.3	10.6	0.7	36.3	29.0	This work
Swine	0	55	20	10.0	Batch	8	29.8	19.3	10.6	1.4	71.5	57.3	This work
Chicken	0.1	65	24.5	8.5	Batch	8	141.7	124.7	17.0	1.1	26.2	20.9	Abouelenien et al., 2009a
Chicken	0.7	37	22.5	8.5	Semi-continuous	~92	107.2	94.4	12.8	0.5	3.4	22.7	Abouelenien et al., 2009b
Chicken	2	35	12.1	7.4	Batch	30	18.8	6.6	12.2	0.3	51.7	~100	Wang et al., 2012
Dairy	2	35	8.0	6.9	Batch	30	8.7	3.0	5.7	0.2	50.2	~100	Wang et al., 2012

*No adjustment.

[a]The data for previous research works were calculated according to the information provided in the related references.

[b]I/S refers to the mass ratio of inocula to substrate (VS basis).

TS-total solid, VS-volatile solid, TKN- total Kjeldahl nitrogen, TON-total organic-N, TAN-total ammonia nitrogen

The variation of TAN at different initial pHs (Figure 2-4c) indicates that the fermentation process is more favorable at initial pH 10.0 than at other tested initial pH conditions, even though a relatively higher FAN might be generated in the fermentation product at initial pH 10.0. This finding coincided with the result obtained by Lin et al. (2013), who reported that initial pH 10 is beneficial for SM solubilization, providing more soluble organic matters accessible to the acidogens and thereby enhanced VFAs and TAN production. At the end of experiments, the ammonia-N yield increased from 7.7 to 11.0 mg/g-VS with the increase in initial pH from 7.0 to 10.0, while declined to 5.5 mg/g-VS when the initial pH was further increased to 12.0. An obvious increase by 44% in organic-N conversion rate or mass conversion ratio was detected when initial pH increased from 7.0 to 10.0, about 1.0 mg/g-VS/d (pH 7.0) to 1.4 mg/g-VS/d (pH 10.0) or 39.8% (pH 7.0) to 57.3% (pH 10.0), respectively. The TAN to TN mass ratio in the SM was largely increased from initial 34.1% to 70.6% after 8 days' ammonia fermentation at initial pH of 10.0.

Like other enzymes, the protease activity depends highly upon system pH. Similar to the trend of TAN production, the protease activity at initial pH 10.0 was significantly higher ($p < 0.05$) than those at other initial pH levels as illustrated in Figure 2-3c. This observation is different from the results of Yu et al. (2008) who detected lower protease activity at pH 10.0 than that at pH 5.5 after 5-20 days' fermentation when activated sludge was used for VFAs production and greatly enhanced VFAs production was achieved when system pH was maintained at 10.0. They attributed the remarkably increased VFAs production at pH 10.0 to enhanced solubilization under alkaline conditions and thus more effective contact between soluble organics and extracellular enzymes (abiotic mechanism). This difference is most probably brought about by the different operation strategies applied in these two research works. More specifically, the system pH was maintained at 10.0 by daily addition of NaOH in the previous study (Yu et al., 2008); while in this work only initial pH adjustment was done before ammonia fermentation, and a decline in system pH (from initial pH 10.0 to 8.5 on day 8) might lead to the gradual increase in protease activity (Figure 2-3c).

Based on the above results, the optimum operation parameters for ammonia release from SM by dry AD in this study were determined as: thermophilic temperature (55 °C)

and 20% TS with initial pH 10.0. The operation conditions obtained in this work can serve as a useful reference for process design in practical applications of dry AD.

Table 2-2 summarizes the conversion efficiencies of organic-N to TAN in four major manure samples under different anaerobic fermentation conditions. Despite the beneficial effects of low TS content on the extent to which organic-N conversion can proceed, high organic-N conversion rates of 36.3-71.5 mg/g-TON/d can be achieved through dry AD at TS $\geq$ 20% and thermophilic temperatures, which are comparable to those obtained at lower TS of 8.0% to 12.1% (Wang et al., 2012). It indicates that dry anaerobic fermentation is a promising and effective method for ammonia production from livestock manure owing to its simple operation, fast organic-N conversion rate, high volumetric ammonia yield and subsequently low construction cost.

2.3.3. Changes of P bioavailability during the short-term dry AD

Figure 2-6a depicts the analytical results of P fractions and pH variations in RSM and the digestates obtained from the 8 days' dry AD at different temperatures. As shown, TP remained stable at around 18.0 mg/g-TS in all tested manure samples. The increase in temperature, however, has some positive effect on the conversion of OP to IP. Generally, the P group in OP compounds is bound to C by an ester bond. Thus OP is mineralized as a byproduct of the C cycle, during which enzymatic hydrolysis is an essential step for the degradation of OP to IP (Tiessen, 2011). Various enzymes such as nucleases, phytase, phospholipase, and phosphatases are involved in this enzymatic hydrolysis process, and the activity of each enzyme depends highly upon system temperature and pH (Tiessen, 2011; Turner et al., 2005). After the 8 days' dry AD process, the proportion of potentially bioavailable P decreased slightly from 61.3% (RSM) to 59.8%-58.6%. This is to some extent consistent with the finding of Güngör and Karthikeyan (2008) who declared that AD of dairy manure was capable of reducing the immediately available P in the solid phases. On the other hand, the system pH dropped from 8.6 to 8.0, 7.8 and 7.7 after dry AD at 25 °C, 35 °C and 55 °C, respectively, most probably resulting from VFAs accumulation (Figure 2-3a).

Results of P fractionation and pH in RSM and the digestates after the 8 days' dry AD at 55 °C and different TS contents are displayed in Figure 2-6b. It was observed that at

the end of experiments, the conversion efficiency of OP increased from 18.4% to 47.4% with TS increased from 20% to 35%. This observation might be partially explained by the increase in their final pHs from 7.7 at 20% TS to 8.3 at 35% TS. A strong correlation relationship was found between the OP conversion efficiency and the final pH of the digestate by using linear regression analysis ($R^2 = 0.9960$). Unlike the production of VFAs, TS content was not the limiting factor dictating the conversion efficiency of OP to IP. Instead, system pH played a more important role in OP mineralization. A proper increase in the system pH might be beneficial for the microbial degradation of OP. In this work a greater extent of OP mineralization seemed to be accompanied by a slightly higher final pH (like 35% TS).

Figure 2-6c displays the effects of initial system pH on P speciation after the 8 days' dry AD at 55 °C and 20% TS. An increase in initial pH from 7.0 to 10.0 contributed to an increased OP conversion efficiency from 15.8% to 68.4%. Further increase of system pH to 11.0 or 12.0, however, led to a decline in OP conversion efficiency. On the other hand, a larger proportion of AP in IP was detected in the digestate when dry AD was conducted at higher initial pHs. This phenomenon was due to the fact that in this study $Ca(OH)_2$ was employed to adjust the initial pH of SM to alkaline conditions under which Ca^{2+} and PO_4^{3-} could react with OH^- to form apatite (Van Kemenade and De Bruyn, 1987), contributing to a higher amount of AP in the digestate. The lowest potentially bioavailable P was detected to be 1.7 mg/g-TS at initial pH of 10.0, accounting for 9.5% of TP in the digestate.

Table 2-3 lists the availabilities of TVFAs, TAN and P species in the digestate after dry AD under 4 typical conditions. In spite of the highest TVFAs and TAN yields obtained in the digestate from dry AD at 55 °C, 20% TS and initial pH 10.0, its bioavailable P (OP + NAIP) was very low. In contrast, after 8 days' dry AD at 55 °C, 20% TS and no adjustment of initial pH, relatively high concentrations of TVFAs, TAN and bioavailable P in the digestate were obtained simultaneously. In order to optimize the costs relating to chemicals consumption and to meet the requirements of multipurpose utilization of the digestate, the optimal dry AD condition was determined as 55 °C, 20% TS and unadjusted initial pH. The digestate obtained after 8 days' dry AD under the optimal conditions underwent dry or wet ammonia stripping; thereafter the availability of C, N and P in the separated liquid solution and solid residue were explored.
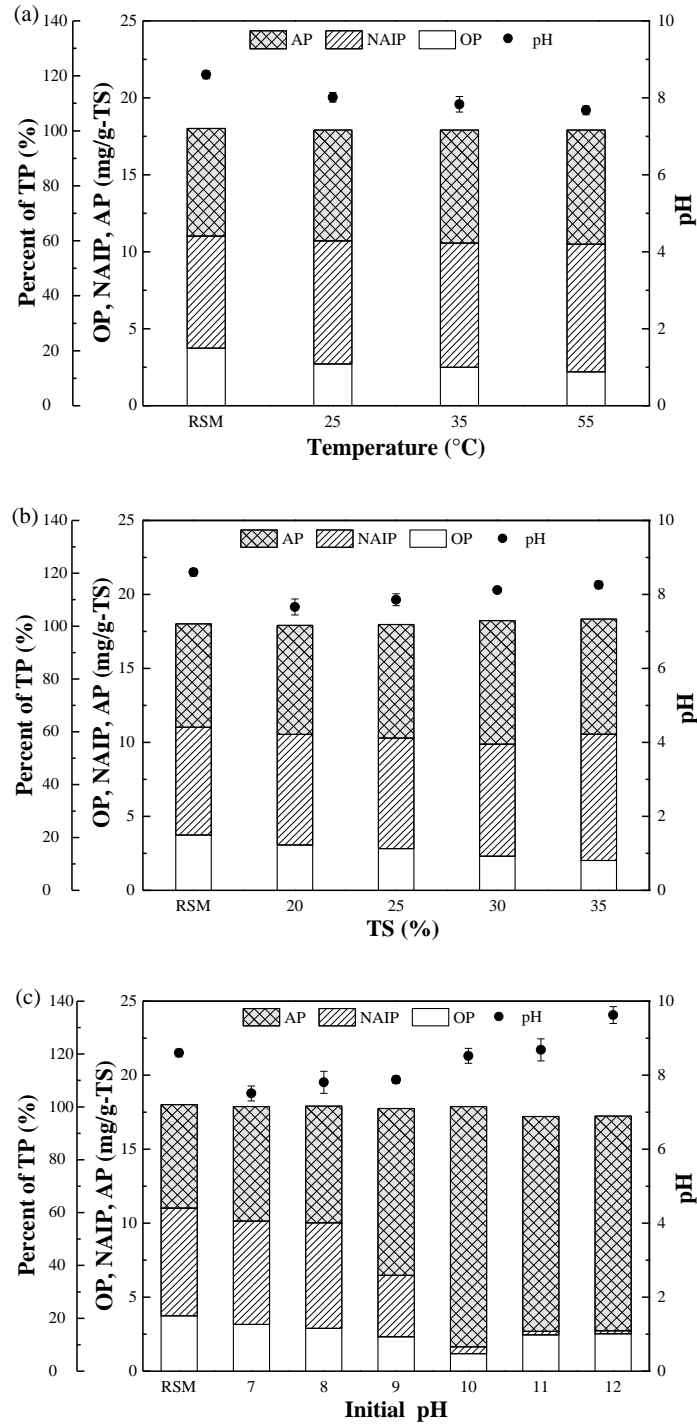
Figure 2-6. Profiles of pH and P fractionation in raw swine manure (RSM) and the digestate under different dry AD conditions tested: (a) operation temperature at 25 °C, 35 °C and 55 °C (25% TS and no initial pH adjustment); (b) TS content of 20-35% (at 55 °C without pH adjustment); and (c) initial pH varied from 7.0 to 12.0 (at 55 °C and 20% TS), respectively.

Table 2-3. Availability of nutrient elements and total volatile fatty acids in the digestate under 4 typical experimental conditions after 8 days' dry AD.

	Fermentation conditions			Compositions of nutrient elements and TVFAs in the digestate						
No.	Temperature (°C)	TS (%)	Initial pH (chemical used)	TVFAs (mg-COD/g-VS)	TAN (mg/g-VS)	TON (mg/g-VS)	OP (mg/g-TS)	NAIP (mg/g-TS)	AP (mg/g-TS)	P bioavailability [b] (%)
1	55	20	8.6[a]	94.4	20.0	9.7	3.1	7.5	7.4	58.9
2	55	35	8.6[a]	67.4	16.2	13.7	2.0	8.5	7.8	57.3
3	55	20	8.0 (HCl)	89.6	19.9	9.9	2.9	7.1	7.9	55.9
4	55	20	10.0 (Ca(OH)$_2$)	114.8	21.3	8.3	1.2	0.5	16.2	9.5

[a]No initial pH adjustment. [b]P bioavailability (%)=100 × (OP + NAIP)/TP.
AP-apatite phosphorus, NIAP-non-apatite inorganic phosphorus, OP-organic phosphorus, TAN-total ammonia nitrogen, TON-total organic nitrogen, TS-total solids, TVFAs-total volatile fatty acids, VS-volatile solids.

2.4. Ammonia recovery and separation of VFAs from P resource

Figure 2-7a displays the removal efficiencies of TAN from the solid digestate under different stripping conditions. An increase in both initial pH and temperature gave rise to higher concentrations of FAN (Hansen et al., 1998), contributing to higher removal efficiencies of TAN from the digestate. The highest TAN removal efficiency of 96.2% was achieved at 55 °C and initial pH 11.0 after 3 hours' stripping. Quantitative analysis of the amount of TAN entrapped by acid solution or water and that escaped from the digestate revealed a high TAN recovery efficiency $\geq 95.5\%$ under all stripping conditions.

As illustrated in Figure 2-7b, most probably as a consequence of ammonia removal the digestate pH dropped in all circumstances, from 10.0 to 8.9 and 8.5, and from 11.0 to 9.6 and 9.1 when the stripping was conducted at 35 °C and 55 °C, respectively. On the other hand, the concentration of TVFAs in the digestate remained pretty stable throughout the whole stripping process. As it is known, VFAs are volatile under acidic conditions while they become stable and exist as ionic forms in alkaline solutions. In the stripping systems, due to the fact that pH was maintained at pH > 7.0, the escaped TVFAs from the reactor were considered insignificant. In these trials, averagely 92.5% of the TVFAs were successfully retained in the digestate after 3 hours' stripping at 55 °C and initial pH 11.0. Only 4.7% and 2.1% of TVFAs were averagely detected in the scrubbing solutions and the water containing bottle (vessel 3 in Figure 2-1b), respectively. And the loss of TVFAs from the stripping system was most probably brought about by measurement errors. Based on the experimental results, the effective TAN removal duration (τ_e), about 60 min and 90 min, was obtained for the stripping process at initial pH 11.0 and pH 10.0 (55 °C), respectively. The former (55 °C and initial pH 11.0) was considered to be the optimal dry ammonia stripping condition in this study.

The removal efficiencies of TAN during wet ammonia stripping are shown in Figure 2-7c. At the end of stripping, the removal efficiencies of TAN were detected to be 79.1% at 35 °C and initial pH 10.0, 91.5% at 55 °C and initial pH 10.0, and 95.0% at 35 °C and initial pH 11.0, respectively. The highest TAN removal efficiency was achieved at 55 °C and initial pH 11.0, which increased rapidly to 98.7% after stripping for 2 hours, and then climbed slowly to 99.7% after 3 hours' stripping. TAN balance analysis again exhibited good performance of TAN recovery by using the stripping/absorption processes. Greater

than 94.0% of the stripped TAN from the filtrate was able to be entrapped by the acid solution and water in all cases studied.

As shown in Figure 2-7d, the filtrate pH declined during the process of stripping from 10.0 to 8.8 (35 °C) and 8.4 (55 °C), and from 11.0 to 9.7 (35 °C) and 9.1 (55 °C), respectively. On the other hand, the decrease of TVFAs in the filtrate was found to be less than 11% in all the tested scenarios. For instance, an average decrease of 6.3% after stripping was detected when the stripping was conducted at 55 °C and initial pH 11.0. Moreover, to a great extent the lost TVFAs could be re-entrapped by the scrubbing solutions (~ 61%) and water containing vessel 3 (~ 32%, Figure 2-1b). Again, based on Figure 2-7c, the effective TAN removal duration (τ_e) was determined to be around 65 min and 85 min, respectively for wet stripping at initial pH 11.0 and pH 10.0 (55 °C), which was almost similar to that of dry ammonia stripping process (Figure 2-7a).
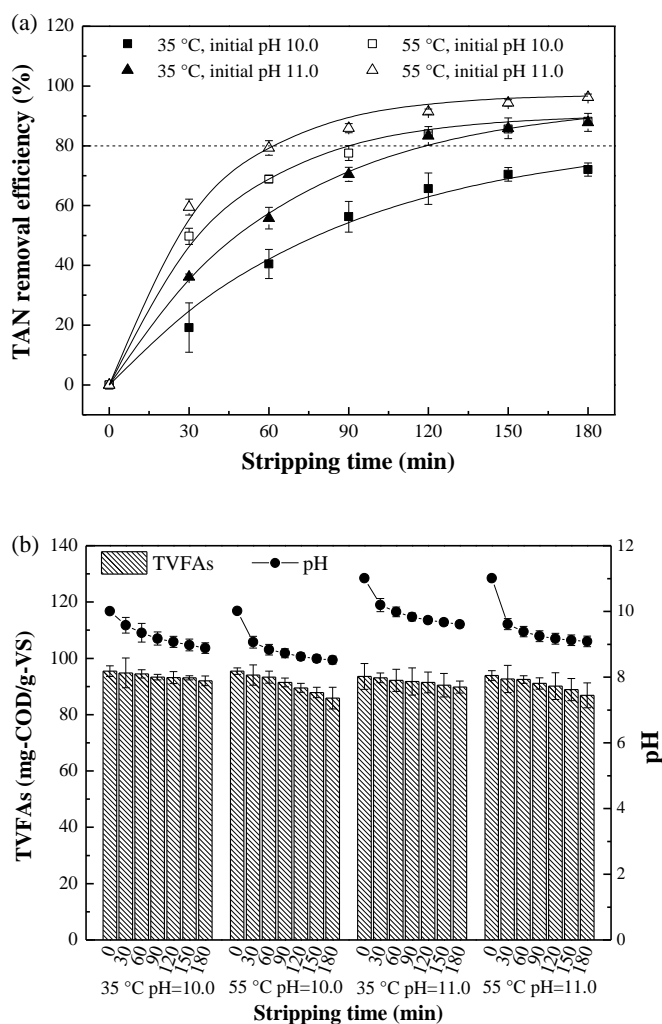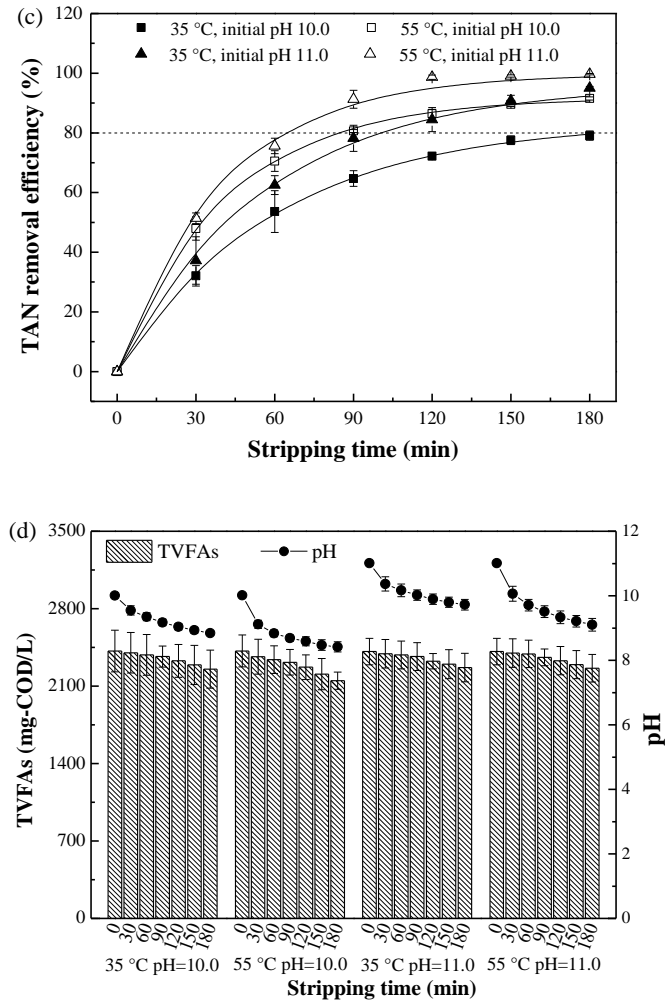
Figure 2-7. Effects of different operational conditions on (a) ammonia removal efficiency and (b) variations of pH and TVFAs during dry ammonia stripping, and those of different stripping conditions on (c) ammonia removal efficiency and (d) changes of pH and TVFAs during wet ammonia stripping.

Two ammonia stripping strategies (dry or wet) were employed for ammonia recovery from the digestate, which were further compared in terms of technical and economic aspects. According to the results of kinetic analysis presented in Table 2-4, the experimental data from both dry and wet ammonia stripping processes fitted well to the pseudo first-order kinetic model (R^2 = 0.9916-0.9997). Interestingly, when operated under the same temperature and initial pH conditions, almost similar A_{eq} values were obtained by either dry or wet ammonia stripping. This observation indicated that TS content could only affect the kinetics of TAN removal since similar A_{eq} values were achieved under both dry and wet stripping conditions while a higher TAN removal rate

constant (k) for wet stripping process. Results indicated that the extent to which ammonia stripping could proceed was driven by the substrate FAN/TAN ratio closely related to system pH and temperature, whereas the ammonia removal rate was largely affected not only by the effective contact between gas and substrate, but also the influencing factors like gas distribution and existing form, viscosity and fluidity of the substrate. Among all the tested scenarios, 55 °C, the optimal temperature for VFAs production, was also found to be the optimal temperature for both dry and wet ammonia stripping in this study. In addition, for these two stripping strategies, about 60-65 min and 85-90 min were necessary for achieving 80% of TAN removal from the digestate or filtrate when ammonia stripping was conducted at 55 °C with initial pH 11.0 and pH 10.0, respectively (Table 2-4).

Table 2-4. Stripping rate constants associated with the pseudo first-order kinetic model and effective TAN removal durations under different stripping conditions.

Strategy	Stripping conditions		Pseudo first-order kinetic model			τ_e (min)
	Temperature (°C)	Initial pH	A_{eq} (%)	k (min^{-1})	R^2	
I: Dry stripping	35	10.0	83.4	0.0117	0.9916	278.5
	55	10.0	90.6	0.0238	0.9970	90.2
	35	11.0	95.2	0.0154	0.9974	119.1
	55	11.0	97.3	0.0280	0.9958	61.7
II: Wet stripping	35	10.0	83.9	0.0165	0.9996	186.0
	55	10.0	91.9	0.0242	0.9997	84.5
	35	11.0	96.4	0.0176	0.9984	100.6
	55	11.0	99.9	0.0251	0.9977	64.3

In the stripping system developed in this study, the gas was circulated among the vessels and acid absorption solutions in a close loop, to a great extent avoiding unnecessary ammonia loss and OH$^-$ consumption resulted from the reaction between NaOH and CO_2 in the air. From this work, dry ammonia stripping possesses the following advantages compared to traditional wet stripping. Firstly, dry ammonia stripping can avoid the foaming problems which always occur in wet systems. Secondly, much higher volumetric TAN removal rates (0.75-0.99 g/L-digestate/h) were obtained in the dry ammonia stripping systems operated at 20% TS, in comparison to those (0.14-0.17 g/L-filtrate/h) of wet ammonia stripping systems. In addition, processing of the digestate in

semi-solid state requires much smaller reactor and thus less construction investment. And thirdly, the consumptions of energy for heating as well as chemicals (i.e. both alkalis applied to raise digestate/filtrate pH during ammonia stripping and acids used for re-neutralization of the ammonia-stripped digestate/filtrate before being further processed) are considerably saved.

Table 2-5. Main characteristics of the final liquid solutions and solid residues obtained from the two operation strategies under their corresponding optimal stripping conditions (both at 55 °C and initial pH 11.0).

	Parameters	Unit	Strategy I (Dry stripping)	Strategy II (Wet stripping)
Liquid phase	Total ammonia nitrogen (TAN)	mg/L	19.5 (±3.3)	1.6 (±1.1)
	Soluble ortho-P	mg/L	93.1 (±4.6)	86.7 (±6.7)
	Total volatile fatty acids (TVFAs)	mg-COD/L	2247.3 (±114.2)	2261.2 (±125.0)
	Soluble chemical oxygen demand (SCOD)	mg/L	3926.6 (±163.6)	3728.5 (±177.9)
	pH	—	9.2 (±0.3)	9.1 (±0.2)
Solid phase	Total solids (TS)	%	17.5 (±1.6)	16.4 (±1.6)
	Volatile solids (VS, TS based)	%	76.3 (±1.0)	77.0 (±1.5)
	Total ammonia nitrogen (TAN)	mg/g-VS	0.5 (±0.0)	4.0 (±0.3)
	Total organic nitrogen (TON)	mg/g-VS	9.1 (±0.3)	9.5 (±0.5)
	Organic phosphorus (OP)	mg/g-TS	2.4 (±0.3)	3.1 (±0.3)
	Apatite phosphorus (AP)	mg/g-TS	7.6 (±0.8)	7.3 (±0.6)
	Non-apatite inorganic phosphorus (NAIP)	mg/g-TS	5.7 (±0.3)	5.3 (±0.2)
	C/N	—	25.7 (±0.6)	21.9 (±0.5)
	pH	—	8.4 (±0.2)	7.6 (±0.3)

The data were expressed as mean (±SD).

Zhang and Chen (2009) stated that it's feasible to use struvite sedimentation for simultaneous recovery of ammonia-N and soluble ortho-P from sludge fermentation liquor. This process, however, is less beneficial for the liquid extract from fermented manure mainly due to the following two considerations. (1) Numerous organic compounds and inorganic ions co-existing in the liquid would definitely affect the purity

of struvite precipitates. (2) The theoretical molecular ratio of N:P in struvite is 1:1, while the molecular ratio of TAN to ortho-P in the liquid extract was close to 13:1. The concentration of water extractable ortho-P (~90 mg/L) in the fermented manure was disproportionally lower than that of TAN (~540 mg/L) due to the fact that most P was fixed in biomass or formed precipitates with the co-existing metallic ions like $Fe^{2+/3+}$ and Ca^{2+} (Huang et al., 2015). In this context, dry ammonia stripping offers a promising alternative for direct separation and recovery of ammonia-N from the dry AD digestate.

Table 2-5 presents the main characteristics of the liquid and solid phases obtained by strategies I and II, respectively, at stripping conditions of 55 °C and initial pH 11.0. As it can be seen, the final concentration of TVFAs was almost same in the liquid phase by using both stripping processes, around 2250 mg-COD/L. As for P availability, slightly higher concentration of soluble ortho-P was detected in the liquid after dry ammonia stripping, possibly due to thermal-alkaline hydrolysis of the organic solids during the stripping process. As expected, a slightly lower concentration of OP was detected in the solid residue after strategy I, about 2.4 mg/g-TS in comparison to 3.1 mg/g-TS of OP in the solid residue acquired with strategy II. AP and NAIP concentrations were almost similar regardless of different stripping strategies applied. In summary, the potentially bioavailable P in the solid residue was able to be maintained at a relatively high level of 8.1-8.4 mg/g-TS (51.6%-53.5% of TP) after ammonia stripping and solid-liquid separation through both strategies. Most notably, the C/N ratios in the solid residues after strategies I and II were detected to be 25.7 and 21.9, respectively, both higher than that of RSM (C/N=18.0, Table 2-1) and falling within the optimal C/N range (20-30) for biomethane production (Esposito et al., 2012).

2.5. Environmental implications

After the two-step treatment process under optimal conditions, the obtained liquid rich in VFAs (Table 2-5) might be utilized as external carbon source to enhance biological nutrients removal after being further processed (like extraction) or used for other industrial purposes. The recovered ammonia, on the other hand, is a valuable raw material that can be used for synthesis of chemical fertilizers (e.g. urea), antibacterial agents and many commercial cleaning products. The solid residue with much lower N content while

maintaining a relatively high content of bioavailable P can serve as feedstock for dry CH_4 fermentation or composting for solid fertilizer production. In brief, it is practically feasible to achieve maximum utilization of C, N and P resources in livestock manure by combining short-term dry AD and dry ammonia stripping technologies.

2.6. Summary

This chapter presented a novel strategy for stabilization and utilization of SM using short-term dry AD followed by dry ammonia stripping, specifically focusing on the changes in VFAs, TAN and P bioavailability throughout the treatment procedure. The high conversion rate of organic-N and preservation of bioavailable P in the SM during dry AD along with the high ammonia recovery efficiency from digested excreta at TS around 20% is of great interest to practical application when construction cost is taken into consideration. Both the recovered ammonia and separated VFAs solution are marketable products.

Chapter 3 Low-temperature hydrothermal pretreatment to improve methane production from dry anaerobic digestion of swine manure and to facilitate nutrient utilization

3.1. Introduction

HT technology is an advantageous choice due to its efficacy, simplicity, no chemical addition, low water requirement and sanitation effects. Although this process has been successfully implemented at full-scale for improving biogas production from animal byproducts, lignocellulosic materials, food wastes and sewage sludge (Carrere et al., 2016), little information is available for its pilot- or full-scale application in animal manure treatment, especially under high TS conditions.

Most studies conducted on HT pretreatment for improving AD process reported an optimal HT temperature range of 170-190 °C (Bougrier et al., 2008; Wilson and Novak, 2009). However, pretreatment under such high temperature levels leads to high energy consumption and strict requirements for the equipment. Another potential disadvantage is the undesirable decrease in system pH/alkalinity under high HT temperature conditions (Yin et al., 2014), and pH adjustment is often required to achieve neutral that is suitable for methanogenesis (Chen et al., 2007), which also needs extra cost for process operation. What's more, a high ammonia released during HT process could exert severe inhibition to the methanogens during AD of the pretreated animal manure. Thus, the application of low-temperature pretreatment might be more beneficial. For instance, Wilson and Novak (2009) suggested a HT temperature < 150 °C in order to marginally reduce ammonia release from pretreated activated sludge. On the other hand, insignificant changes in sludge biodegradability and degradation rate were noticed by Dwyer et al. (2008) when the HT temperature was decreased from 165 to 140 °C. After the mechanically separated solid animal manure being HT pretreated at 120 °C for holding 30 min, Menardo et al. (2011) obtained 35%-171% increase in CH_4 yield from the pretreated biomass (TS = 12-28%). Despite these efforts, more details regarding energy balance, microbial dynamics and the possible

reclamation of nutrient elements are necessary to fully assess the stability and reliability of low-temperature HT pretreatment for improving dry AD of manure waste. Particularly, fertilizer potential of the digestates related with existing forms of N and P — the two most important nutrient components in commercial fertilizers, is also important for evaluating the economic performance of a biogas plant.

The aim of this study was to investigate the feasibility of dry AD of animal manure after HT pretreatment at low temperatures of 110 and 130 °C. Restated, CH_4 production from dry AD of the pretreated SM and the changes in N and P availability were studied. In addition, energy balance analysis was conducted to compare the energy input for pretreatment (heating and stirring) and the net increase in energy output (improvement in CH_4 yield). Finally, the whole process performance of low-temperature HT pretreatment followed by dry AD was assessed by comparing the net energy yield, C capture efficiency and fertilizer potentials of the digestate.

3.2. Materials and methods

3.2.1. Raw materials

Table 3-1. Characteristics of the raw swine manure and inocula used in this study.

Parameter	Raw swine manure (RSM)	Inocula
Total solids (TS, %)	25.02 ± 0.11	19.10 ± 0.21
Volatile solids (VS, %)	20.06 ± 0.12	11.74 ± 0.15
Total alkalinity (TA, mg/g as $CaCO_3$)	18.34 ± 0.53	26.66 ± 0.64
Total ammonia nitrogen (TAN, mg/g)	0.62 ± 0.02	2.59 ± 0.10
Total nitrogen (TN, mg/g)	4.67 ± 0.15	3.54 ± 0.14
Organic phosphorus (OP, mg/g)	1.12 ± 0.09	0.70 ± 0.04
Apatite phosphorus (AP, mg/g)	3.80 ± 0.21	5.36 ± 0.31
Non-apatite inorganic phosphorus (NAIP, mg/g)	2.13 ± 0.14	1.47 ± 0.08
C/N	16.76 ± 0.20	13.10 ± 0.17
pH	6.91 ± 0.08	7.54 ± 0.08

*The results are presented as mean values of five determination ± standard deviation. All concentrations in the table were calculated based on fresh matters.

RSM was sampled from a pig finishing unit near Tsukuba campus, where the fattening pigs were fed with soybean, corn, bran, etc. Fresh manure that contained no

bedding materials was collected by a shovel from concrete floor of the pig house. The collected RSM was mixed thoroughly and refrigerated at 4 °C before use. Mature digestate that sampled from a semi-batch dry AD reactor treating SM taken from the same farm was used as inocula. The semi-batch reactor was successfully operated at 35 °C for about 5 months in the lab after acclimation. Table 1 lists the characteristics of the RSM and the inocula used in this study.

3.2.2. Hydrothermal treatment

An enclosed stainless steel reactor (cylindrical shape with inner diameter 4.0 cm, height of 19.0 cm, and wall thickness of 0.25 cm) equipped with an agitator (OM Lab-tech MMJ-200, Japan) was employed for the HT experiments. In each HT experiment, the reactor was loaded with 140 g RSM (TS 25% without dilution) and sealed. Under constantly stirring at 100 rpm, the reactor was heated up at an average rate of 14.4 °C/min to a designated temperature (110 and 130 °C) and maintained at the temperature for 30 min. The corresponding pressures at 110 and 130 °C was 0.05 and 0.18 MPa, respectively. Then the heater was powered off and the reactor vessel was cooled with a fan to ambient temperature (around 25 °C) before opened for sampling. All the HT experiments were conducted in triplicate, and the analysis of each parameter was repeated twice. After sample analysis, 100 g manure sample was taken from each HT runs (totally 300 g for each HT test condition) and used for the subsequent dry AD experiment.

3.2.3. Dry anaerobic digestion

For CH_4 fermentation, 300 g RSM or pretreated SM at 110 and 130 °C were mixed thoroughly with 150 g inocula, then the mixtures were loaded into a series of 500 ml fermentation bottles, labeled as R-RSM, R-110 and R-130, respectively. Meanwhile, a fermentation bottle loaded with inocula only was prepared as the control. The bottles were then flushed with N_2 for 2 min to create an anaerobic environment, sealed with rubber stoppers and incubated at 35 ($\pm$ 1) °C for 70 days without agitation. To simplify

the process for its possible application in practice and to avoid changes in the microenvironment brought about by acid/alkali addition, no further pH adjustment was performed. The initial AD conditions are listed in Table 3-2. All analyses of the SM characteristics were performed three times. The biogas ($H_2 + CH_4 + CO_2$, ml/g-VS) was collected and quantified, and the gas compositions (H_2, CH_4 and CO_2) was determined with a Shimadzu GC (Japan) equipped with thermal conductivity detector. Net CH_4 yield (in ml/g-VS fed) of the SM was obtained by subtracting the amount produced from the inocula.

3.2.4. Sample analysis

Sample preparation and analysis of the related parameters including TS, VS, manure pH, TN, organic C and N contents, TAN, VFAs, and P components (OP, NAIP and AP) were performed in accordance with the procedures described in Chapter 2.2.4. Total alkalinity (TA) was determined by titration method to a pH end point of 4.3. Soluble proteins and carbohydrates were analyzed with Coomassie brilliant blue method (Bradford, 1976) and phenol-sulfuric method (Herbert et al., 1971), respectively. Amino acids were quantified by an amino acid analyzer (JEOL JLC-500/V2, Japan). Soluble organic carbon (SOC) was determined by a TOC-V$_{CSN}$ (Shimadzu, Japan).

Table 3-2. Changes of soluble organic products and nutrient components in the manure samples before and after dry AD.

Day	Reactor	pH	TA as CaCO$_3$ (mg/g)	TS (%)	VS (%)	C/N	SOC (mg/g)	TAN production		P fractionation			
								TAN (mg/g)	TAN/TN (%)	TP (mg/g)	OP/TP (%)	NAIP/TP (%)	AP/TP (%)
	R-RSM	6.96	21.04	22.97	17.23	15.70	7.80	1.09	27.32	7.29	13.59	26.24	60.17
0	R-110	6.66	20.88	22.88	17.17	13.84	8.45	1.30	32.45	7.33	12.78	27.05	60.18
	R-130	6.61	20.93	22.72	17.06	13.75	9.06	1.35	34.20	7.32	11.41	24.45	64.15
	R-RSM	7.54	21.52	19.27	12.07	11.54	5.26	1.56	37.84	9.02	7.24	15.65	77.10
70	R-110	7.65	21.27	17.89	11.33	11.55	5.64	1.94	47.12	9.17	8.88	18.12	72.99
	R-130	7.67	21.36	17.73	11.08	11.70	8.68	2.87	70.67	9.33	7.80	15.41	76.80

TA, SOC, TAN and TP were based on fresh matters.

TA-total alkalinity, AP-apatite phosphorus, NAIP-non-apatite inorganic phosphorus, OP-organic phosphorus, SOC-soluble organic carbon, TAN-total ammonia nitrogen, TN-total nitrogen, TS-total solids, VS-volatile solid.

3.2.5. Calculations and kinetics

Effective biogas production duration (τ_e, d) defined as the fermentation duration for achieving 80% of the total biogas production and averagely effective biogas production rate (r_e, ml/g-VS/d) calculated by Eq. 3-1 were used to indicate the AD performance of each reactor.

$$r_e \text{ (ml/g-VS/d)} = 80\% \text{ of the total biogas yield (ml/g-VS)}/\tau_e \text{ (d)} \qquad \text{3-1}$$

To assess anaerobic biodegradability of the SM samples and to predict the AD performance, a curve-type kinetic model (Eq. 3-2) was applied for process analysis. The model has been successfully employed in a previous study regarding anaerobic digestibility of pig manure pretreated by thermal steam explosion (Ferreira et al., 2014).

$$Y = Y_m \cdot \{1 - \exp[-r_m(t - \lambda)/Y_m]\} \qquad \text{3-2}$$

where Y the accumulated biogas yield (ml/g-VS), Y_m the theoretical maximum biogas yield (ml/g-VS), r_m the maximum biogas production rate (ml/g-VS/d), λ the lag period (d) and t the fermentation duration (d).

3.2.6. Energy assessment

To evaluate the feasibility and scalability of HT pretreatment, energy assessment was conducted based on the experimental results obtained in this work. Input energy was calculated as sum of the energy required to heat the SM from ambient temperature (T_a) to the preset hydrothermal temperature (T_{HT}) and the energy consumed for mixing during the HT process, which could be subtracted by the amount recovered by heat exchanger during the cooling process, with an efficiency of 85% (Lu et al., 2008). The following assumptions were made in this work: (1) the SM used was homogeneous with a specific heat capacity C (kJ/kg/°C) and density ρ (kg/m^3) similar to that of water; (2) the ambient temperature was constant at 25 °C; (3) insulation materials were used and the heat radiated during HT reactions was not taken into consideration; and (4) no mass was lost during the HT process. Energy input was estimated according to Eq. 3-3 modified from that proposed by Passos and Ferrer (2015).

$$Q_{input} = \rho V C (T_{HT} - T_a) + \omega(t_{rise} + t_{hold}) - \xi \rho V C (T_{HT} - T_d) \qquad \text{3-3}$$

Where Q_{input} (kJ) the input energy for HT experiment, C (kJ/kg/°C) heat capacity, ρ (kg/m^3) SM density, V (m^3) the volume of SM for a single HT experiment, ω (kJ/s) power of the mixing device, t_{rise} (s) time elapsed for temperature rise from ambient to designated temperatures, t_{hold} (s) holding time at designated temperatures, T_a (°C) ambient temperature, T_{HT} (°C) preset hydrothermal temperature, T_d (°C) the dry AD temperature and ξ (%) energy recovery efficiency by heat exchanger.

The energy output was calculated from CH$_4$ production (P, m^3/kg) of the dry AD system (Eq. 3-4).

$$\Delta Q_{output} = \eta \rho V q \Delta P \qquad\qquad 3\text{-}4$$

where ΔQ_{output} (kJ) the improvement in energy output, q (kJ/m^3) lower heating value of CH$_4$, ΔP (m^3/kg) the increase in CH$_4$ yield from the HT pretreated SM relative to that from RSM, and η (%) the efficiency of energy conversion, which was assumed to be 90% according to Passos and Ferrer (2015).

Finally, as indicated in Eq. 3-5, energy balance ΔQ (kJ) was arrived at by comparing ΔQ_{output} and Q_{input}. All the parameters used for energy assessment were summarized in Table 3-3. (Supplementary Information).

$$\Delta Q = \Delta Q_{output} - Q_{input} \qquad\qquad 3\text{-}5$$

3.2.7. Statistics

All concentrations in Chapter 3 were calculated based on fresh matters unless otherwise indicated. All results from the HT experiments were presented as mean values of six repeated measurements (3 triplicate HT experiments × 2 repetitions of sample analysis). During dry AD, results of the solid characteristics were calculated as mean values of three repetitions of the analysis. The statistics was analyzed by using Microsoft Excel 2013, and significant difference was assumed at $p < 0.05$.

Table 3-3. Parameters for energy assessment.

Parameters	Unit	Value
Ambient temperature (T_a)	°C	25
HT temperature (T_{HT})	°C	110, 130
Digestion temperature (T_d)	°C	35
Density of SM/water (ρ)	kg/m^3	1000
Specific heat capacity of SM/water (C)	kJ/kg/°C	4.18
Volume of SM for a single experiment (V)	m^3	1.4×10^{-4}
Lower heating value of CH$_4$ (q)	kJ/m^3	35800
Heat recovery by heat exchanger (ξ)	%	85
Energy conversion efficiency of CH$_4$ (η)	%	90
Rated power of the mixing device (ω)	kJ/s	1.5×10^{-2}
Time elapsed for temperature rise from ambient to designated temperatures (t_{rise})	s	372, 468
Holding time at designated temperature (t_{hold})	s	1800

3.3. Results and discussion

3.3.1. Hydrothermal pretreatment
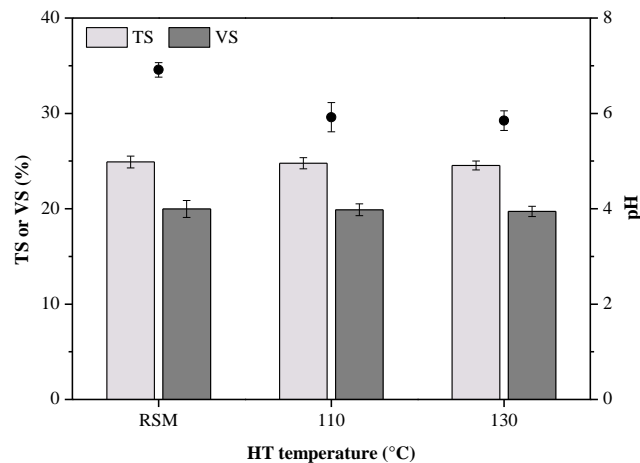
Figure 3-1. Changes in total solids (TS), volatile solids (VS) and system pH after the hydrothermal (HT) treatment.

After HT pretreatment, the decrease in TS and VS was insignificant (p = 0.1823-0.1037 > 0.05) at both HT temperatures as indicated in Figure 3-1. The system pH decreased from initial value of 6.91 to 5.92 at 110 °C and to 5.85 at 130 °C. Further decrease in pretreated manure pH was observed when HT temperature was increased

from 110 to 130 °C, signaling the formation of more organic acids and free hydrogen ions at 130 °C.

Table 3-4. Components of amino acids in RSM and the HT pretreated swine manure samples.

No.	Amino acids	Formula	Concentration ($\times 10^{-3}$ mg/g)		
			RSM	110 °C	130 °C
1	Phosphatidylserine	$C_{13}H_{24}NO_{10}P$	81.88	113.56	134.28
2	Anserine	$C_{10}H_{16}N_4O_3$	ND	ND	1.85
3	Carnosine	$C_9H_{14}N_4O_3$	ND	ND	ND
4	Cystathionine	$C_7H_{14}N_2O_4S$	3.54	3.61	4.03
5	Tyrosine	$C_9H_{11}NO_3$	1.78	8.29	9.64
6	Citrulline	$C_6H_{13}N_3O_3$	3.64	6.30	6.50
7	Arginine	$C_6H_{14}N_4O_2$	0.33	5.06	5.57
8	3-methylhistidine	$C_7H_{11}N_3O_2$	ND	ND	ND
9	Phenylalanine	$C_9H_{11}NO_2$	0.66	6.68	7.91
10	Hydroxylysine	$C_6H_{14}N_2O_3$	18.63	18.40	19.90
11	Histidine	$C_6H_9N_3O_2$	ND	4.61	5.94
12	Methionine	$C_5H_{11}NO_2S$	0.66	3.19	3.66
13	Glutamic acid	$C_5H_9NO_4$	3.90	63.92	72.61
14	Lysine	$C_6H_{14}N_2O_2$	1.83	22.40	26.53
15	Aspartic acid	$C_4H_7NO_4$	1.85	20.95	23.91
16	Ornithine	$C_5H_{12}N_2O_2$	0.21	3.76	4.15
17	iso-Leucine	$C_6H_{13}NO_2$	6.38	12.32	14.62
18	Leucine	$C_6H_{13}NO_2$	8.11	17.98	21.11
19	Taurine	$C_2H_7NO_3S$	ND	8.50	10.05
20	Phenethylamine	$C_8H_{11}N$	1.79	5.02	6.19
21	Cysteine	$C_3H_7NO_2S$	0.41	0.82	1.10
22	Threonine	$C_4H_9NO_3$	0.63	5.92	6.68
23	Valine	$C_5H_{11}NO_2$	14.46	17.20	22.31
24	Proline	$C_5H_9NO_2$	ND	5.55	7.20
25	Serine	$C_3H_7NO_3$	1.09	5.04	6.28
26	α-Aminobutyric acid	$C_4H_9NO_2$	4.21	3.98	4.85
27	β-Aminobutyric acid	$C_4H_9NO_2$	2.62	4.40	5.78
28	γ-Aminobutyric acid	$C_4H_9NO_2$	1.39	7.39	9.57
29	β- Alanine	$C_3H_7NO_2$	2.72	4.21	4.68
30	Sarcosine	$C_3H_7NO_2$	ND	3.31	2.67
31	Alanine	$C_3H_7NO_2$	20.91	38.35	47.14
32	Glycine	$C_2H_5NO_2$	0.79	5.92	8.15
33	Ethanolamine	C_2H_7NO	ND	1.29	2.39
Sum			184.41	427.91	507.28

*ND-not detected.
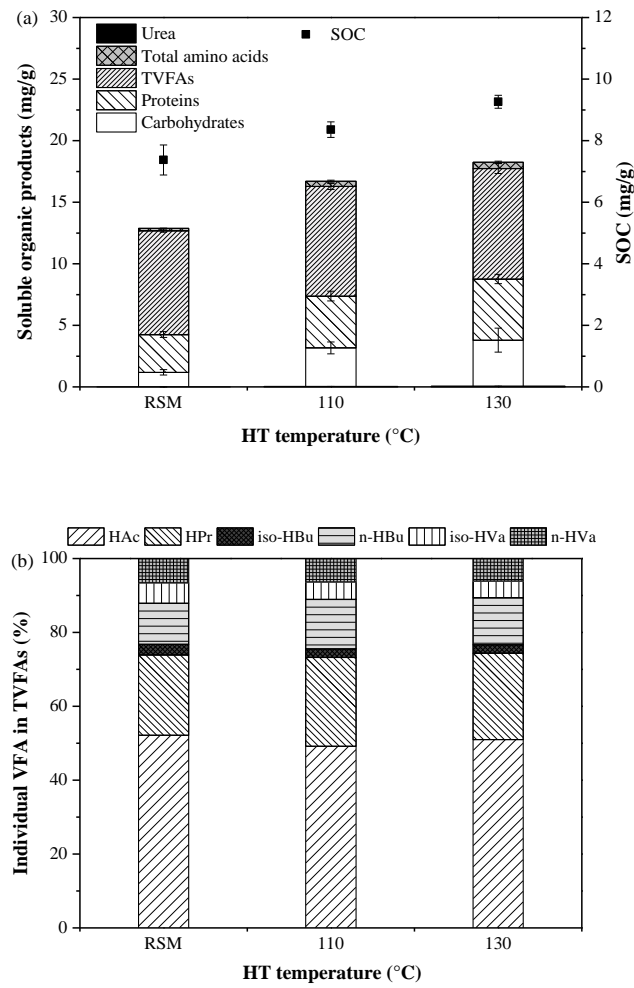
Figure 3-2. Changes of soluble organic products (a), and percentage distribution of VFAs (b) in the manure samples. Soluble organic carbon (SOC), volatile fatty acids (VFAs), raw swine manure (RSM), acetic acid (HAc), propionic acid (HPr), iso-butyric acid (iso-HBu), n-butyric acid (n-HBu), iso-valeric acid (iso-HVa) and n-valeric acid (n-HVa), hydrothermal (HT).

Figure 3-2a illustrates the effect of reaction temperatures on the productions of SOC and some major soluble organic compounds including TVFAs, carbohydrates, proteins, total amino acids and urea. After HT pretreatment at 110 and 130 °C, the SOC concentration increased from 7.37 to 8.36 and 9.26 mg/g respectively. An increase in HT temperature from 110 to 130 °C was beneficial for the accumulation of soluble proteins and carbohydrates. As shown, the soluble proteins increased by 36.92% and 61.66% in the SM pretreated at 110 and 130 °C, respectively. Compared to proteins, HT temperature exerted more pronounced influence on the variations of soluble

carbohydrates. A carbohydrates concentration of 3.17 and 3.80 mg/g was recorded respectively at 110 and 130 °C, resulting in an increase by 170% and 220% when compared to that in RSM (1.18 mg/g), respectively.

HT pretreatment on SM at 110-130 °C showed little impact on the production of TVFAs ($p = 0.4281 > 0.05$) which remained relatively stable at 8.5-9.0 mg/g (Figure 3-2a). This phenomenon is in agreement with the results observed by Wilson and Novak (2009), who stated that VFAs was yielded mainly from hydrothermal degradation of lipid, proteins and amino acids at temperatures over 170 °C. Little change in VFAs composition was also observed when HT pretreatment was conducted at 110 and 130 °C (Figure 3-2b). Acetic acid (HAc) was the dominant species responsible for 49%-52% of TVFAs in all the tested SM samples (Figure 3-2b).

A total of 33 kinds of amino acids were detected from the HT pretreated SM in this study as summarized in Table 3-4. The concentration of total amino acids increased from 0.18 mg/g in RSM to 0.43 and 0.51 mg/g after HT pretreatment at 110 and 130 °C, respectively. Only trace of urea (≤ 0.05 mg/g) was detected from the pretreated manure samples although it seemed to increase with the increase in temperature from 110 to 130 °C.

Complex organic N compounds can be mineralized mainly to ammonia through hydrothermal reactions (He et al., 2015), and the produced ammonia is recognized as an important inhibitor for methanogens at high solid state (Rajagopal et al., 2013). As indicated in Figure 3-3a, TAN accounted for 13.23% of TN in RSM, and this proportion increased to 17.40% and 18.13% when HT pretreatment was conducted at 110 °C and 130 °C, respectively.
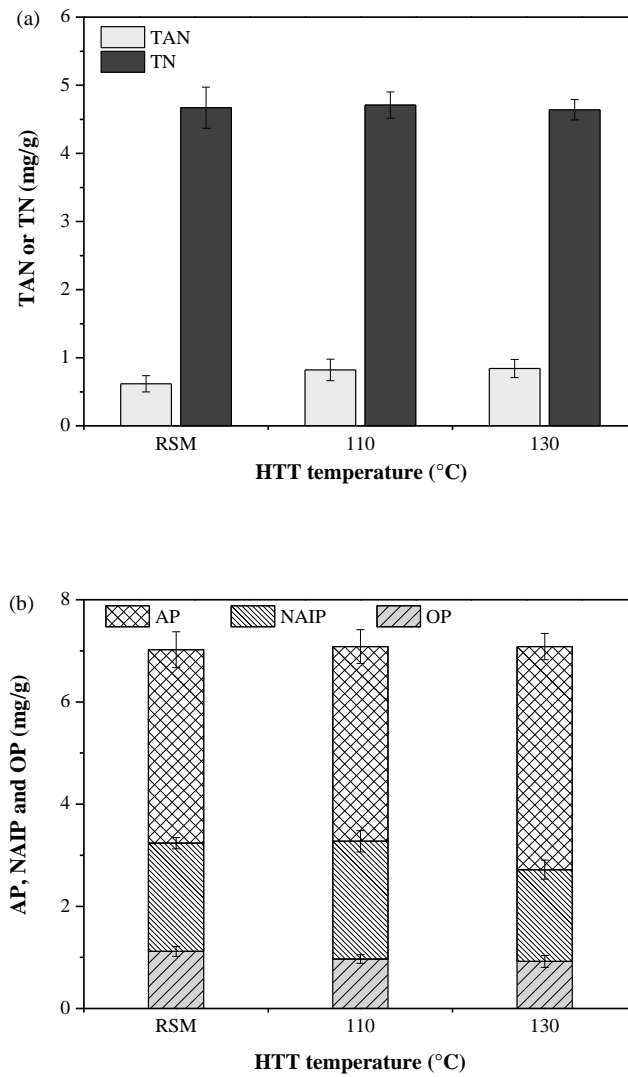
Figure 3-3. Concentrations of (a) TAN and TN, and (b) phosphorus species in the raw and the processed swine manure under different HT temperatures. Total ammonia nitrogen (TAN), total nitrogen (TN), apatite phosphorus (AP), non-apatite phosphorus (NAIP), organic phosphorus (OP), hydrothermal (HT).

NAIP refers to the fraction of P associated with Al, Fe and Mn hydroxides (or oxides), whereas AP is the Ca-bound P that is hard to be utilized by plants or microbes (Ruban et al., 1999). OP is a complex organic fraction that includes phospholipids, nucleic acids and phytates (Uchimiya and Hiradate, 2014). The term potentially bioavailable P generally refers to the fractions possible to be released and assimilated by organisms, i.e. OP + NAIP. As shown in Figure 3-3b, the distribution of P species in the SM was slightly influenced by HT pretreatment. The OP content in RSM was 1.12

mg/g, accounting for 15.92% of its TP. This OP content was found to reduce to 0.97 and 0.92 mg/g after SM being pretreated at 110 and 130 °C, about 13.69% and 13.02% of TP, respectively. 46.09% of the TP content in RSM was potentially bioavailable, this proportion remained almost unchanged after SM being HT pretreated at 110 °C, while it decreased to 38.36% after HT pretreatment at 130 °C (Figure 3-3b). Hydrothermal disintegration of the metal-complexing organic manure may set free the multivalent metal cations which then become movable, possibly enabling the effective contact between calcium and the resultant P from OP decomposition and NAIP dissociation. A higher HT temperature is more beneficial for the disintegration of organic matters, thus promoting the formation of non-bioavailable AP. In this work, HT pretreatment at 110 °C showed no significant influence on AP content in SM ($p = 0.9253 > 0.05$), attributable to its low degradation efficiency of organics in manure waste.

3.3.2. Dry anaerobic digestion of the pretreated swine manure

As shown in Table 3-2, the initial pH in R-RSM, R-110 ad R-130 was 6.96, 6.66 and 6.61, respectively, which fell within the narrow pH range (6.6-7.6) suitable for methanogens to function (Chen et al., 2007). The initial TA in these three reactors varied between 20.88 and 21.04 mg/g as $CaCO_3$. Apparently, HT pretreatment at 110-130 °C did not bring about significant decrease in SM pH or alkalinity, which made it possible to directly implement dry AD of the pretreated SM (without further pH adjustment).

Figure 3-4 exhibits the changes in daily biogas yield, accumulated biogas yield and CH_4 content in the reactors during 70 days' operation. As shown, after a short period of adaptation, biogas started to evolve quickly from reactor R-RSM, R-110 and R-130 (Figure 3-4a), achieving accumulated biogas yields of 300.99, 352.04 and 411.59 ml/g-VS_{fed} on day 70, respectively (Figure 3-4b). The application of well-acclimated inocula in this study contributed a lot to this fast start-up and process stabilization. And since the inocula has been pre-exposed to high concentrations of ammonia, retarded or suppressed CH_4 production by ammonia inhibition was not observed in this work. The effective biogas production duration (τ_e) for R-RSM was 42 d, with averagely effective

biogas production rate (r_e) of 5.73 ml/g-VS/d. Compared to RSM (R-RSM), HT pretreatment at 110 °C (R-110) slightly shortened τ_e to 39 d while significantly increased r_e to 7.23 ml/g-VS/d. When HT pretreatment was conducted at 130 °C, a longer τ_e of 46 days and an improved r_e value of 7.16 ml/g-VS/d were obtained during the subsequent dry AD (R-130).

Table 3-5 presents the kinetic parameters obtained by fitting Eq. 3-2 to the experimental data on accumulated biogas yield. It can be seen that low-temperature HT pretreatment at 110-130 °C could contribute to improved biogasification from SM. The estimated maximum biogas yields (Y_m) are in agreement with the experimental data. Nevertheless, the gaps between the predicted Y_m values and the experimental biogas yields may be attributed to the complexity of dry AD process and heterogeneity of SM. To achieve the maximum CH_4 recovery from dry AD of manure wastes, further work is necessary for process optimization like reactor configuration design and mixing conditions, etc.

Table 3-5. Kinetic parameters for different dry AD reactors.

Reactor	τ_e (d)	r_e (ml/g-VS/d)	Measured Y on day 70 (ml/g-VS)	Kinetic model			
				Y_m (ml/g-VS)	λ (d)	r_m (ml/g-VS/d)	R^2
Control (inocula)	-	-	87.60	91.09	1.86	8.72	0.9578
R-RSM	42	5.73	300.99	439.34	2.83	8.15	0.9872
R-110	39	7.23	352.04	487.98	2.16	11.14	0.9853
R-130	46	7.16	411.59	685.46	3.47	10.16	0.9939

As shown in Figure 3-4c, a two-phase methanogenesis was discerned from R-RSM, R-110, and R-130. The CH_4 content in R-RSM, R-110, and R-130 increased rapidly at the beginning to a maximum of around 70% on day 6-7, then it hit the bottom on day 8, day 11 and day 15, respectively, followed by a gradual rebound to 70%-75%. The trend of CH_4 content in biogas might signal a gradual shift in CH_4 production pathway from hydrogenotrophic to aceticlastic methanogenesis. The accumulated CH_4 yield from R-RSM, R-110 and R-130 on day 70 was 203.50, 230.81 and 270.52 ml/g-VS,

corresponding to a net CH$_4$ yield (corrected from the inocula) of 246.03, 280.18 and 328.93 ml/g-VS$_{fed}$, respectively.
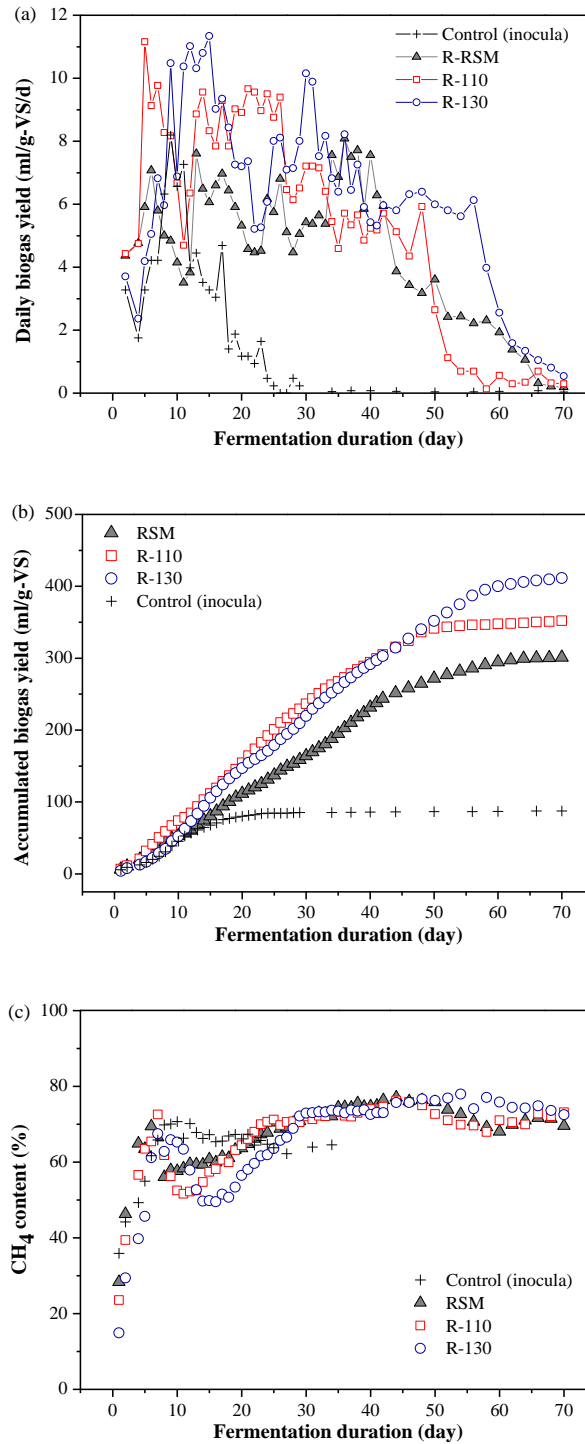

Figure 3-4. Daily biogas production (a), accumulated biogas yield (b) and CH$_4$ content (c) during the fermentation process.

As shown in Table 3-2, after dry AD, significant decrease in VS content by 29.95%, 34.01% and 35.05% was detected respectively in R-RSM, R-110, and R-130. On day 70, the concentrations of TVFAs, proteins and carbohydrates in the three reactors was detected to be low (Figure 3-5). The concentrations of these soluble organic products are mainly governed by two factors: (1) the activity of methanogens for converting the soluble organics into CH_4, and (2) the hydrolysis rate of solid manure, which depends highly upon its biodegradability (Veeken and Hamelers, 1999). As indicated by the plateaued CH_4 production in the late stages of dry AD (after day 65) and the low contents of soluble organic products detected in R-RSM, R-110 and R-130 at the end of the experiment, the decreased hydrolysis rate is most probably brought about by the exhaustion of easily biodegradable organics in these reactors.
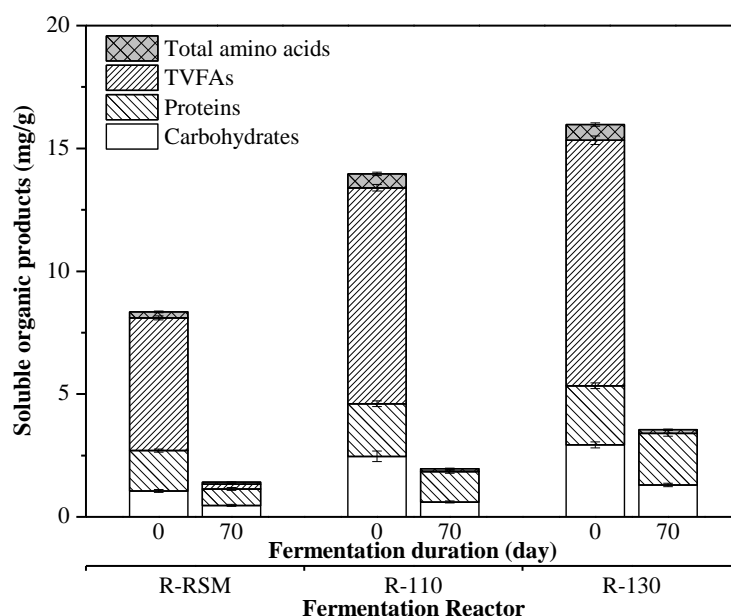
Figure 3-5. Soluble organic products in the reactors before and after 70 days' dry AD (anaerobic digestion) experiment. Total volatile fatty acids (TVFAs).

The above results show that HT pretreatment at 110-130 °C can improve CH_4 production from SM, resulting in greater extent of organic manure stabilization. This is important for agricultural application of the digestate since unstable digestate containing labile organics could generate problems like immobilization of inorganic

nutrient elements, pathogens re-growth, odor/CO_2 emission, and reduced N availability via denitrification (Alburquerque et al., 2012).

Economic benefits of dry AD include not only the biogas produced, but also the fertilizer potential of digestate related with the available macronutrients (like N and P) and micronutrients (like amino acids). In this section, the changes of N and P availability in SM during the dry AD were investigated. As listed in Table 3-2, the incremental effect of dry AD on manure TAN/TN ratio was in a descending order of R-RSM (+ 10.52%) < R-110 (+ 14.67%) < R-130 (+ 36.47%). Generally, AD leads to degradation of labile organic compounds and increase of ammonia-N concentration (Alburquerque et al., 2012; Tambone et al., 2010), which is important since the N fraction available to plants is closely related to inorganic N content of the digestate. HT pretreatment at 130 °C greatly promoted organic-N mineralization of manure samples during dry AD, possible due to the increased overall anaerobic degradability of the pretreated SM.

P fractionation in manure samples before and after dry AD is also displayed in Table 2. The content of TP increased from 7.29 to 9.02 mg/g in R-RSM, from 7.33 to 9.17 mg/g in R-110 and from 7.32 to 9.33 mg/g in R-130, respectively as a result of effective VS reduction of SM during the biogasification process. After 70 days' dry AD, the proportions of NAIP and OP in the resultant digestates decreased, whereas those of AP increased, suggesting that available P was reduced through the formation of stable Ca-bound P during dry AD. At the end of dry AD, potentially bioavailable P was statistically unaffected by HT pretreatment at 110-130 °C, about 23-27% detected in all the manure samples. Since most of the labile organics were degraded after dry AD, P existing forms in the digestate were mostly determined by the total multivalent metal ions (Ca^{2+}, Mg^{2+}, Fe^{2+}, Al^{3+}, etc.) content in the original SM and their relative stoichiometric relationship between each other.

3.3.3. Energy balance and comparison analysis

Base on the experimental results obtained, energy balance was analyzed to justify the economic feasibility of low-temperature HT pretreatment for improving dry AD of SM in terms of net energy gain. In this study, the input energy for a single HT trial (140 g substrate) at 130 °C was calculated to be 48.22 kJ, while the improvement in output energy was 75.02 kJ. Thus, a net energy increase of 26.80 kJ (corresponding to 0.95 kJ/g-VS) was gained by adopting HT pretreatment at 130 °C. For HT pretreatment at 110 °C, a negative net energy gain of -0.50 kJ/g-VS was obtained.

Table 3-6 compares the performances of different treatment strategies for the utilization of solid manures. As shown, composting is a simple and effective process for animal manure stabilization, however, a high proportion of C, N and P is lost during manure composting. For insect farming, there are some technical challenges that need to be overcome like insect breeding and collection. Both pyrolysis and combustion are fast and effective physico-chemical methods to convert organic manure into energy. However, due to the high water content in animal manure, large amount of energy is required for manure drying before pyrolysis or combustion. Also, the extreme reaction conditions call for high requirements for facilities. Compared with other effective treatment strategies, low-temperature HT pretreatment followed by dry AD obviously possesses merits like relatively milder reaction conditions, high recovery efficiency of organic C as biogas, minimum loss of nutrients, and no chemical consumption.

3.3.4. Implications of the findings

The two most urgent challenges restricting the rapid development of human society are energy shortage and growing demand for food supply. Specifically in Japan, since the 2011 great earthquake, the safety and reliability of nuclear power has been questioned, and renewable energy is attracting more and more attention. On the other hand, Japan relies heavily on the import of inorganic fertilizers (mainly N and P) from other countries to guarantee enough food production. In order to sustain the country's economic development and increase the profits and efficiency of agricultural industry,

Japan must secure a stable and reliable supply of both energy and nutrient resources. For these reasons, interest has grown increasingly in the utilization of biomass wastes. Particularly, the large amount of SM generated nationwide (22 million tonnes per year) (MAFF, 2015) represents an abundant resource for energy recovery and nutrient elements recycling. Currently, however, 99.7% of the solid SM fractions obtained via solid-liquid separation are treated by composting (MAFF, 2011). Shortcomings of this practice are emission of greenhouse gases, loss of N through ammonia volatilization and significant decrease in P availability (Christel et al., 2014; Tambone et al., 2010). The implementation of dry AD facilitated by low-temperature HT pretreatment can achieve high bioenergy recovery efficiency and a marketable final product (digestate) with good fertility due to its high retention of N and P nutrients in available forms. The bioenergy and digestate obtained are good candidates to substitute fossil fuels and inorganic fertilizers, respectively.

3.4. Summary

Low-temperature HT pretreatment was evaluated for improving dry AD of the SM. According to the experimental results, HT pretreatment at 130 °C contributed to: (1) improved CH_4 yield by 33.70% during 70 days' dry AD of the pretreated SM without extra pH adjustment; (2) 38% of the organic-C in SM being recovered as valuable CH_4, (3) a positive net energy gain of 0.95 kJ/g-VS; and (4) accelerated organic-N mineralization, with 70.67% of TN in the digestate existed as ammonia-N.

Table 3-6. Different treatment strategies to utilize solid animal manures.

Manure	Treatment method	Chemical used	Reaction period	Energy yield [a]	C recovery	N recovery	P recovery	Total amino acids	Other final products	Reference
Swine	Dry AD	—	70 days	19.7 m^3 CH$_4$ (7.1×10^5 kJ) per 100 kg dry mass	28 % of organic-C recovered as CH$_4$ while 5% released as CO$_2$	38% of TN recovered as TAN in the digestate	23% of TP remained bioavailable in the digestate	82.1 µg/g	Digestate as fertilizer	This study
Swine	HT pretreatment (130 °C for holding 30 min) followed by dry AD	—	~ 70 days	26.4 m^3 CH$_4$ (9.5×10^5 kJ) per 100 kg dry mass	38% of organic-C recovered as CH$_4$ while 7% released as CO$_2$	71% of TN recovered as TAN in the digestate	23% of TP remained bioavailable in the digestate	154.9 µg/g	Digestate as fertilizer	This study
Swine	Unturned windrow composting (mixed with cornstalk)	—	42 days	—	42%-54% of organic-C was lost as CO$_2$	40%-60% of TN was lost	20%-38% of TP was lost	Not reported	Compost as fertilizer	Tiquia et al., 2002
Swine	Turned windrow composting (mixed with cornstalk and turned once a week)	—	42 days	—	60%-68% of organic-C was lost as CO$_2$	37%-59% of TN was lost	23%-42% of TP was lost	Not reported	Compost as fertilizer	Tiquia et al., 2002
Swine	Insect rearing and biodiesel production	C$_7$H$_7$BrMg, CH$_3$OH and KOH	6 days for larvae growth and 0.5-2 days for drying	1.9 L biodiesel per 100 kg dry mass fed to the larvae	Not reported	Not reported	Not reported	Not reported	Degreased larvae meal and residues as fertilizers	Yang and Liu, 2014
Dairy	Combustion at 900 °C	—	Maintained at 125 °C for 10 min, and heated at a rate of 10 °C/min	1.2 ×10^6 kJ per 100 kg dry mass	Energy in organic-C was released with CO$_2$ and CO as exhaust gas	Not reported	Not reported	—	Ashes rich in P and K	Fernandez-Lopez et al., 2015

Dairy	Pyrolysis at 1000 °C	—	Maintained at 125 °C for 10 min, and heated at a rate of 10 °C/min	$3.6×10^2$ kJ per 100 kg dry mass	Most organic-C was converted into CO_2 and CO	Not reported	Not reported	—	—	Fernandez-Lopez et al., 2015

[a] Energy recovery presented in the table do not take the energy input for pretreatment/operation into consideration. TN-total nitrogen, TP-total phosphorus, HT-hydrothermal, TAN-total ammonia nitrogen.

Chapter 4 Conclusions and future research

4.1. Conclusions

In this thesis, short-term dry AD followed by air stripping and low-temperature HT pretreatment were applied respectively in an attempt to enhance dry AD of SM and nutrients utilization. Conclusions regarding the first treatment strategy can be drawn as follows:

(1) Thermophilic temperature, lower TS of 20% and moderate alkaline initial pH of 8.0-10.0 are beneficial for the accumulation of VFAs and ammonia during short-term dry AD of SM. After 8 days' dry AD under above conditions, high volumetric TVFAs and TAN production rates of 979.7-1468.6 mg-COD/L/d and 181.3-214.0 mg/L/d were achieved, respectively.

(2) In the thermophilic dry AD system, proper increase in system pH (brought about by increasing TS from 20% to 35%) was beneficial for the microbial degradation of OP. Mineralization of OP was found to be significantly enhanced when SM was fermented under 20% TS and 55 °C by using $Ca(OH)_2$ to adjust initial pH, which lowered the amount of bioavailable P (OP + NAIP). Considering full utilization of C, N and P resources in the SM, the short-term dry AD without initial pH adjustment (pH~8.6) is suggested in practice.

(3) The two-step procedure involving short-term dry AD and dry ammonia stripping not only provides an alternative for the production and separation of VFAs from ammonia and P resources, but also serves as pretreatment to reduce ammonia buildups. Thus the treated SM residue is also expected to have high potential for dry CH_4 fermentation.

On the other hand, by studying the performance of low-temperature HT pretreatment at 110-130 °C on enhanced SM digestibility and the changes of nutrients bioavailability, the following conclusions can be arrived at:

(1) HT pretreatment at 130 °C contributed to 33.70% increase in CH_4 production during dry AD of SM without extra pH adjustment, as well as high TAN/TN ratio of 70.67% in the final digestate. This temperature was considered optimal in this study.

(2) The compositions of P were statistically unaffected by the HT pretreatment with a potentially bioavailable P fraction of 22.89-27.00% being observed in all reactors. The content of multivalent metal ions in the starting SM was the sole determining factor.

(3) Net increase of 0.95 kJ/g-VS in the energy output was achieved by adopting HT pretreatment at 130 °C. Low-temperature HT pretreatment followed by dry AD can serve as a simple and sustainable strategy for manure waste stabilization and utilization.

4.2. Future research

To make full use of livestock manure, future research should be followed up in several aspects:

(1) Further enhancement of VFAs production from dry AD of SM and dry CH_4 production of the resultant solid residue. Specifically, in addition to cost-effectiveness analysis of the whole system, attentions should also be paid to the preservation and retention of the bioavailable N and P resources in the digestate and utilization of the digestate as high quality fertilizers as well.

(2) Further optimization of ammonia stripping and acid scrubbing technology (e. g. using screw type propeller and countercurrent packed absorption tower) to timely remove and recover ammonia from swine excreta during dry ammonia fermentation is also crucial for ammonia/VFAs production, ammonia recovery, and subsequent biogas production.

(3) Chemical properties of the digestate obtained after HT pretreatment and dry CH_4 fermentation should be further characterized. And the potential phytotoxicity, hygiene as well as biological stability of the digestate need to be fully evaluated.

(4) Pilot scale experiments should be conducted to fully evaluate the practicability of HT pretreatment for improving dry AD of SM in terms of economic and environmental aspects.

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