Characteristics of Anaerobic Fermentation by Rotational and Fixed Media Reactors

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Characteristics of Anaerobic Fermentation by Rotational and Fixed Media Reactors

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CONTENTS

Abstracts

Waste from food processing contains abundant of organics, which are easy to pollute the environment and should be well treated. In fact, the organics in waste from food processing can be recovered and converted to bioenergy, which have been paid attention by many researches and governments. The anaerobic fermentation of food waste has been focused on due to the simultaneous waste reduction and bioenergy recovery. The most crucial factors for anaerobic fermentation are considered as the characteristics of sludge and substrates. The substrates for anaerobic fermentation mainly included the solid and liquid waste. For solid waste, the high substrate concentration and high recalcitrant organic contents make the hydrolysis become the rate-limiting step, and caused the acidogenic products accumulation, which seriously inhibited anaerobic process. For the liquid food processing waste, the high nitrogen concentration and low C/N ratio are important problems. The liquid food processing waste usually experiences the nitrification and denitrification, and the element of nitrogen is released in the form of gas rather than recovered. Therefore, it is necessary to develop bio-reactors and processes for the anaerobic fermentation of solid waste and liquid waste from food processing, respectively.

The objectives for this work are:(1) For solid waste from food processing, to establish a simultaneous pretreatment and acidogenesis process for effective anaerobic treatment; (2) For liquid waste from food processing with low C/N ratio, to establish a

bioreactor to effectively treat the bulky wastewater, and develop an alkali-free process to recover nitrogen at the optimal operation conditions.

For the solid food processing waste, a simultaneous pretreatment and acidogenesis process was developed by adding methanogenic leachate and andesite porphyry (WRS) powder to a rotational drum fermentation system to enhance the anaerobic fermentation of solid food wastes. In the continuous operations, methanogenic leachate recirculation significantly increased hydrolysis rates and volatile solids (VS) degradation. The VS degradation ratio and the hydrolysis rate constant at a higher leachate recirculation ratio (2:1 weight ratio of methanogenic leachate to substrate) were increased by 2.1- and 1.4-fold, respectively, compared to those of the lower ratio (1:1 leachate recirculation ratio). A 10% (weight ratio of WRS to substrate solid content) WRS addition assisted the biochemical reactions in the process at the higher leachate recirculation ratio was employed. The hydrolysis rate constant and VS degradation were elevated by 54.7% and 63.9%, respectively, with the WRS addition. Besides, the WRS addition enhanced the VA formation and its conversion to biogas

For the liquid food processing waste, a bioammonia production system is developed with fixed bed fermentors by the anaerobic fermentation. Little research has focused on the kinetic analysis of ammonia/ammonium production in anaerobic fermentation. Therefore, in this work, ammonia and ammonium production were simulated by Monod model. The optimal operation condition was determined as 1002

mg-N/L at hydraulic retention time (HRT) of 0.9 d. The microorganism wash-out was predicted to occur at HRT of 0.6 d. These results suggested that the high loading rate of organic nitrogen could be disposed by the developed anaerobic fermentation system.

As is known, ammonia recovery from anaerobic fermentation is mainly dependent on three factors, i.e. ammonium concentration, pH and temperature in the broth. pH adjustment is widely used to remove ammonia in the anaerobic broth. pH is usually adjusted by lime or NaOH in researches, however, alkali addition greatly increased the operation cost in practical engineering. In addition, alkali addition would hamper the consequent methanogenesis due to excessively high cation concentrations and destroy the reaction balance between organic degradation and methanogenesis. In this work, an alkali-free anaerobic system with partial heating modules was developed to enhance bioammonia production and recovery. Partial heating module is composed of a ribbon heater, a thermocouple and a temperature controller. Single and dual partial heating modules were investigated, demonstrating that the single partial heating modules prevailed over the control (without partial heating module). The highest ammonia volatilization rate and total carbon removal ratio obtained in one of the single partial heating modules were elevated by 5.0-folds and 57.9 % compared to the control, respectively.

Abbreviation

ARP	ammonia recovery potential
AVR	ammonia volatilization rate
HRT	hydraulic retention time
IVA	ionized volatile acid
MD	mean diameter
RDF	rotational drum fermentor
SBK	surface based kinetics
SPA	simultaneous pretreatment and acidogenesis
TAN	total ammonium nitrogen
TC	total carbon
TDS	total dissolved solids
TN	total nitrogen
TS	total solids
TSS	total suspended solids
TVA	total volatile acid
UVA	unionized volatile acid
VA	volatile acid
VS	volatile solids
VSS	volatile suspended solids
WRS	wheat-rice-stone (andesite porphyry)

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Chapter 1

Introduction

1.1. Background

The secure, reliable, clean and socially equal energy supply is essential to global economic growth and human development. In the past one hundred years, human have created the great civilization rapider than any periods in histories since the industry revolution, however, the abuse of fossil fuel returns the fuel crisis along with the environment downturn. Many international organizations and government agencies have shown that the fossil fuel production will reach the maximum no later than 2030 (Nel and Cooper, 2009; Leggett and Ball, 2012), and meant that the fossil fuel cannot meet the demands for human activities except the substantial energy price oscillation, which challenges the secure and sustainable energy supply for increasing economy and population. Simultaneously, the greenhouse gas mainly from fossil fuel accumulates in the atmosphere and lead to the significant climate change. The greenhouse gas emission continuously increases from 280 ppm before industry revolution to current approximately 397 ppm, and is keeping an increasingly fast

growth as shown in Figure 1-1 (Dlugokencky et al., 2014).

Considering the sustainable development, renewable energy, such as solar energy, biomass energy, hydropower and ocean energy is becoming a good alternative to replace the fossil fuel in future with merits of environmental friendly and cost-efficient. In addition, the biomass energy is carbon neutral. In comparison with the combustion of fossil fuel, carbon from biomass is derived from the atmosphere instead of fossil bed and the energy reserved in biomass is formed from solar. The biomass energy formation is illustrated by Figure 1-2 (Abbasi and Abbasi, 2010). Biomass mainly originated from the food production and processing, forestry processing and municipal waste (Field et al., 2008; Cucek et al., 2012). Food waste is a typical kind of biomass produced from the food production and processing with characteristics of high organic contents, which means the potential of efficient recovery of bioresource and bioenergy. As for its wide origination and a great deal amount, increasing attention on the food waste treatment and conversion technologies are paid by researches, governments and companies.

1.2. Technical routes for from food waste recovery

Large varieties of energy can be derived from food waste. The biomass and bioenergy conversion routes can be achieved by chemical, biochemical and

thermochemical methods. Beside pyrolysis and hydrothermal, the most wide used thermochemical route for food waste conversion is incineration, but the incineration of food waste also needs improvement to reduce the toxic emission. Biochemical conversion route for biomass energy, such as gasification, esterification, alcohol fermentation and anaerobic fermentation, attracts people as well for the low energy input and mild conditions during the conversion.

1.2.1 Pyrolysis and hydrothermal liquefaction

Food waste can be converted to solid, liquid and gaseous fraction by pyrolysis through pyrolysis in absence of oxygen. The solid fraction is mainly char and ash (inorganic substance), and the liquid and gaseous fraction are able to be prepared as the biofuel. Pyrolysis gas can be used more readily, either in spark ignition or compression ignition engines; however, the nitric oxide reduction techniques are desirable (Hossain and Davies, 2013). Slow pyrolysis is actually used by centuries to produce char at a lower reaction temperature with long time. In comparison, the fast pyrolysis at 500 $^{\circ}$C with a shorter residence time(Jens F. Peters et al., 2014) could produce liquid and gas fuel. However, the fast pyrolysis requires the feedstock to be supplies as fine particles, which limited its utilization and efficiency.

Hydrothermal liquefaction is able to obtain biofuel in under the condition of

250-550 ˚C and 5-25 MPa (Akhtar and Amin, 2011). Not only certain reactors should be provided, but also fine control of the reaction condition is necessary for the hydrothermal liquefaction. It is because various compounds are formed during the hydrothermal liquefaction process depending on operating conditions.

1.2.2. Esterification

Esterification has been recently studied to transfer the bio-oil (mainly from the plants oil processing and the fishing waste) in the form of biodiesel (Takisawa et al., 2013). Direct use of bio-oil is not applicable to most of actual due to the high viscosity (Cho et al., 2012), corrosiveness and oxygen content (Hu et al., 2013). Esterification is the reaction process by means of which triglyceride molecules react with an alcohol in the presence of a catalyst to form esters and glycerol. The catalyst presence is necessary to increase the reaction rate and the esterification reaction yield. Many researches are concentrating on the efficient and cost-effective catalysts for the esterification of bio-oil.

1.2.3. Alcohol Fermentation

The technology of fermentation has been widely used to produce ethanol. In the past decade, bio-ethanol from corn, sorghum and potatoes has suspended because

of the competency with food. On the contrary, ethanol produced from food waste, which contains hemi-cellulose, cellulose and lignin, receives more attentions recently. Specific yeasts are necessary for the ethanol production in by fermentation. Besides, the operation conditions such as temperature, pH and retention time should be controlled subtly in order to obtain a high ethanol yield.

1.2.4. Anaerobic fermentation

Anaerobic fermentation has been well investigated for decades due to its contribution on both bioenergy production and environment production. On one hand, anaerobic fermentation of organics is able to not only produce bioenergy, such as hydrogen and methane, but also effectively dispose the waste/wastewater especially from food production and processing. In comparison with aerobic digestion, anaerobic fermentation prevailed with merits of lower energy consumption, reduction of greenhouse gas emission and recovery of bioenergy and valuable byproducts.

1.3 Factors of anaerobic fermentation of food waste

Anaerobic fermentation of food waste is mainly dependent on the characteristics of substrates and the microorganisms' community species and structure. Contents and compositions of food waste as the substrates vary greatly considering

their originations. The anaerobic fermentation of waste from food processing is also affected by operation conditions, such as the temperature, retention time, the C/N ratio of substrate and the intermediate products.

1.3.1. Substrates source

(1) Solid waste from food processing

Solid waste from food production and processing is mainly composed of hemi-cellulose, cellulose, lignin, protein and lipids and other recalcitrant organics. These organics primarily exit in the form of solid waste. The solid organic wastes are at first hydrolyzed, during which the recalcitrant substrate are converted to small molecules and ready to be further degraded. After the subsequent acidogenesis and methanogenesis, organic substrates are converted to biogas. Many researches have proven that the hydrolysis of solid waste is the rate-limiting step for the anaerobic process.

In order to accelerate the hydrolysis of solid substrates, pretreatment of the recalcitrant wastes is in need. Previous studies have witnessed that the pretreatment of solid substrate effectively improved the hydrolysis and consequently accelerated the whole rate of anaerobic fermentation. Various pretreatments have been well conducted, such as the mechanical pretreatment, chemical pretreatment and the biochemical

pretreatment.

(2) Liquid waste from food processing

Large bulk of liquid waste is charged from food production and processing, which mainly contains soluble organics. Wastewater from food processing is diluted because a great deal of water is used to clean the apparatus and sets during the manufacture. Compare to the solid food waste, the large amount of food wastewater is a significant challenge for its anaerobic treatment. Many studies have concentrated on the development of efficient bio-reactor for this kind of wastewater.

Besides the large bulk, the unbalanced C/N ratio is another problem for the anaerobic fermentation of wastewater from food processing. The organic concentration of wastewater from food processing is not as high as the solid waste from the food production, however, the C/N ratio is generally very low especially for the wastewater from dairymanufacture. Therefore, the development of an efficient bio-reactor for the large bulk of wastewater with low C/N becomes a focus for the treatment of wastewater from food processing.

1.3.2. Temperature

Many researches have documented the significant of effects of temperature on microbes' species and structure, the degradation of organic carbon and nitrogen, and

the biogas yields. Mesophilic and thermophilic operations are most widely used during the anaerobic fermentation. Thermophilic condition is believed to be more effective for organic degradation, including the carbon and nitrogen conversion. It has been proved that the enzymes for hydrolysis of organics were more active at the thermophilic conditions, compared to the operation at mesophilic conditions. Many researches also reported that higher methane yield was obtained at the thermophilic conditions. However, studies also demonstrated that the thermophilic temperature for the whole anaerobic process resulted in an unstable biogas production. This should be partly ascribed to the imbalance between rapid acidogenesis and relatively slow methanogenesis. Long term researches pointed out that the optimum temperature for overall methanogenesis was 35-37 ˚C (Castillo et al., 2006; Chae et al., 2008). This is because that the optimum temperature for most methanogens is around 35-40 ˚C, only minorities of methanogens preferred the the thermophilic temperature at the range of 50-60 ˚C (Ward et al., 2008). In addition, overall thermophilic condition means high energy consumption and more operation cost, which limited its application in practical engineering.

Besides the thermophilic and mesophilic condition, the hyperthermia condition has receiving increasing notice. It has been tried in the northern region to pursue a relatively effective treatment with lower consumption.

1.3.3. Pretreatment

Pretreatment is used to pre-dispose the substrate in order to increase the degradability of recalcitrant organics. It is necessary for the anaerobic process when the substrate originated from the solid waste of food processing, which contains a great deal of lignin, cellulose and other persistent organics. An effective pretreatment should meet several requirements such as producing reactive group for enzymatic attack, avoiding the possible inhibition and toxic for enzymes, microbes and subsequent reactions, minimizing the the demand of energy, chemicals and re-construction of reactor merely intended for pretreatment, and producing few residues(Taherzadeh and Karimi, 2008).

Pretreatment for anaerobic fermentation includes the physical, chemical and biochemical methods. Mechanical pretreatment is a kind of physical pretreatment, which could reduce the particle size of the substrate and increase the substrates' degradability. Compared to pretreatment by the addition of chemicals (such as acid and alkali or enzymes), mechanical pretreatment could avoid the introduction of acid or alkali, which possibly influence the subsequent process of acidogenesis and methanogenesis. Mechanical pretreatment consumes less energy and cost in comparison with thermochemical pretreatment and thus is widely used currently.

However, the specific reactor for mechanic pretreatment is necessary, which

will cause the increase of operation cost. From this perspective, the development of a bio-reactor or process for simultaneous pretreatment and anaerobic process is looked forward to.

1.3.5. Intermediate products

The main intermediate products during the anaerobic fermentation of organics are volatile fatty acids (VFA). VFA is originated from the acidogenesis of organics during the anaerobic process, which is ready to be degraded to the biogas by methanogens. VFA accumulation always occurs in the anaerobic fermentationanaerobic fermentation of solid waste, because of the high organic loading rate and the poor rheological properties. The accumulation of VFA will cause the pH decrease and the increase of unionized volatile acid, which could permeate the microbes' membrane and lead to the inactivation of microbes. In another word, the VFA accumulation may result in the failure of anaerobic fermentationanaerobic fermentation.

Many efforts have made to alleviate the inhibition caused by VFA accumulation, such as the adsorption (Ho and Ho, 2012; Palatsi et al., 2012) by absorbents, dilution by water (Gan et al., 2008), rain or leachate (Lu et al., 2013), changing the feeding pattern (Palatsi et al., 2009) and pre-acclimation of microbes.

1.3.6. C/N ratio and nitrogen degradation

C/N ratio is a key factor for the anaerobic fermentation for both the solid waste and the wastewater. C/N ratio reflects the carbon and nitrogen content of the substrate, which are used as basic nutrients for the microbes. The optimum C/N ratio for anaerobic fermentation is believed to be at the range of 20-30(Li et al., 2011). In fact, C/N considerably varies in different types of wastewater and always very low in the wastewater from food processing because of high protein content. The low C/N ratio not only affects the assimilation of microbes, but also results in a high ammonium/ammonia concentration. The ammonium/ammonia are released from the protein degradation, and considered as the inhibitor for the anaerobic fermentation. The accumulation of ammonium/ammonia significantly affects the methanogenesis and even the whole anaerobic process. The free ammonia is able to permeate the microbes' membrane, cause the imbalance between intracellular and extracellular concentration and result in the inactivity and cell fracture. In addition, methanogens have less tolerance to ammonia than the microbes for hydrolysis and acidogenesis (Angelidaki and Ahring, 1993). Therefore, Ammonia reduction and removal during the anaerobic fermentation have been well investigated.

Many researches have reported the methods for ammonia removal, such as stripping with the addition of alkali, the adsorption by zeolite, and the struvite

precipitation. The ammonia stripping is widely used because it not only effectively reduces the ammonia concentration in the broth, but also is able to achieve the recovery of biomass resource in the form of ammonia. However, the addition of alkali limited the application of ammonia recovery since it considerably increased the operation cost. What's more, the introduction of excessive cation (mainly Na^+, K^+ and Ca^{2+}) is toxic for the microbes. As to the adsorption and the struvite precipitation, the desorption of ammonia becomes another problem. In a word, an alkali-free process to recover ammonia is necessary for a sound and feasible anaerobic fermentation

1.4. Objectives

According to the analysis above mentioned, it is necessary to develop bio-reactors and processes for the anaerobic fermentation of solid waste and liquid waste from food processing, respectively. Therefore, the objectives for this work are:

(1) For solid waste from food processing, to establish a simultaneous pretreatment and acidogenesis process for effective anaerobic treatment (discussed in chapter 2);

(2) For liquid waste from food processing with low C/N ratio, to establish an bioreactor to effectively treat the large bulk wastewater (shown in chapter 3), and develop an alkali-free process to recover nitrogen at the optimal operation conditions

(present in chapter 4).

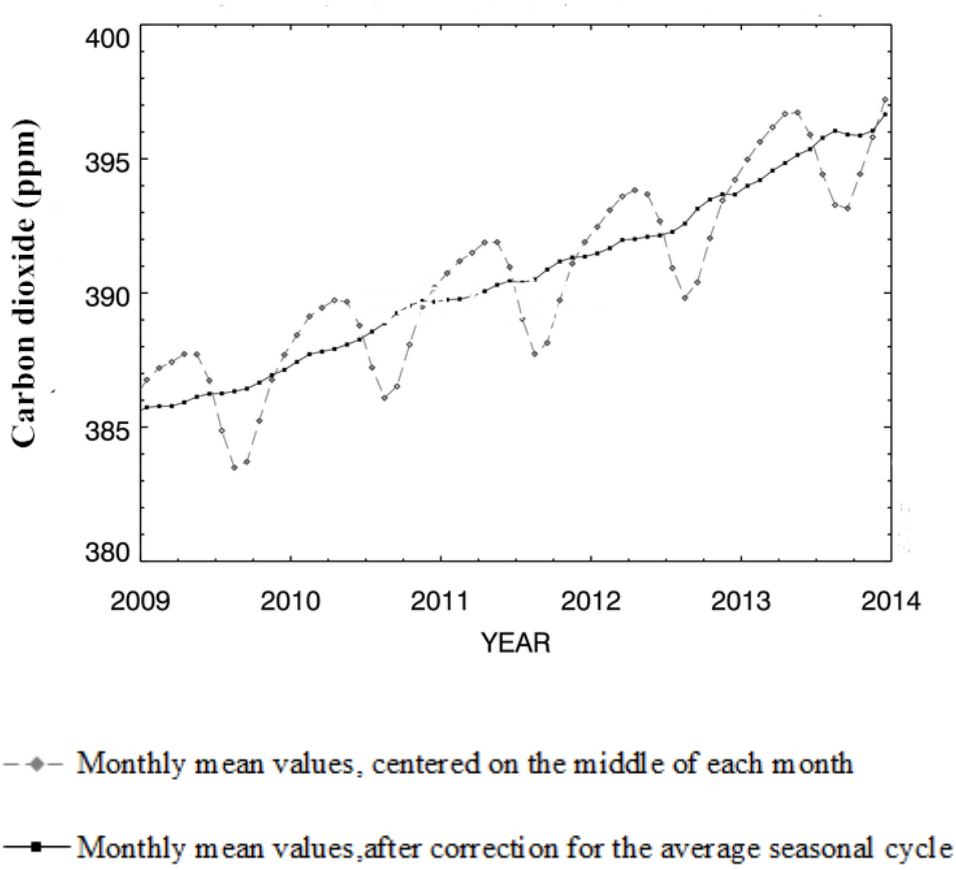
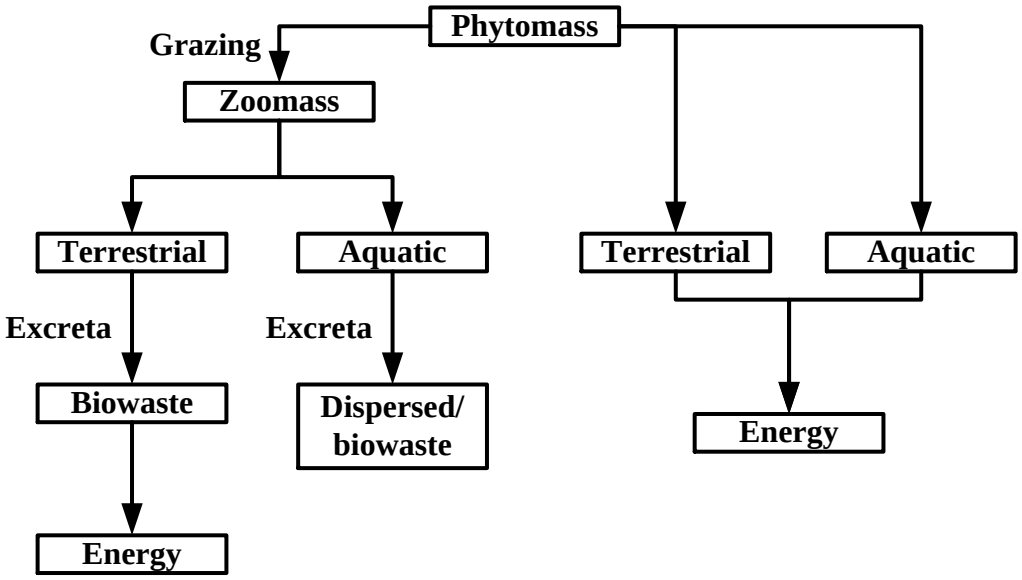


Figure 1-1 Monthly mean carbon dioxide globally averaged over marine surface sites in the period of 2009-2014.



(Abbasi and Abbasi, 2010)

Figure 1-2 The solar energy–biomass energy pathways.

Chapter 2

Characteristics of a simultaneous pretreatment and acidogenesis system for the solid waste from food processing

2.1. Introduction

Bioenergy from solid organic wastes is an excellent alternative to traditional fossil fuels when considering greenhouse gas emission control. Solid food wastes are amenable to bioenergy recovery by anaerobic conversion due to their large biodegradable fractions and moisture. The anaerobic conversion of solid food wastes to the end products CH_4 and CO_2 proceeds in a series of complex biochemical steps due to the lignin content of the solid food wastes (Converti et al., 1999). Hydrolysis is the rate-limiting step in the overall anaerobic conversion of solid substrates (Fdez-Guelfo et al., 2011). The hydrolysis rate mainly depends on the biodegradability of the substrate and the availability of microbes/enzymes (Veeken and Hamelers, 1999), and influenced by many other factors such as rheological properties (Kedziora et al., 2006).

Pretreatments by mechanical, chemical, thermal and combined treatments have been used for decades in the food industry and for biofuel production. Pretreatment contributes to both the reduction of particle size and the rearranging/breaking of some

chemical bonds (McIntosh and Vancov, 2011; Seehra et al., 2012). Ball milling is traditionally applied as a mechanical pretreatment for its ability to rapidly reduce particle sizes and m Among the different ball milling pretreatments, wet milling is preferred to dry milling due to the higher pulverization efficiency (Charkhi et al., 2010) and lower energy consumption (Fuerstenau and Abouzeid, 2002) of wet ball milling. In the wet ball milling process, the pulverization is usually enhanced by increasing the moisture of the feedstock or by adding extra water (Fuerstenau and Abouzeid, 2002). Wet ball milling also meets the requirements for pretreating solid food wastes for anaerobic fermentation.

In the anaerobic fermentation process, the addition of a methanogenic process leachate instead of extra water during the wet ball milling process is conducive to water conservation (Shahriari et al., 2012). Using leachate recirculation to enhance the anaerobic fermentation of solid food wastes (Chen et al., 2008) resulted in a similar VA yield (0.13 g-VA/g-VS) to that (0.11 g-VA/g-VS) of water flushing (Gan et al., 2008). Leachate recirculation improves the rheological properties of substrates and supplies enzymes and microbes to the pretreatment process. The enzymes and microbes also biochemically pretreat the solid substrates. Recycled leachate lowers the acidogenic product concentrations and buffers its inhibition to the hydrolysis and acidogenesis processes as the biochemical pretreatment occurs (Sponza and Agdag, 2004). Pretreatment using leachate recirculation (Zhang et al., 2009) and wet ball milling has been reviewed recently (Chen et al., 2007).

Although wet ball milling with leachate recirculation enforces the stabilization of food waste, the acidogenic products are prone to accumulation when low recirculation ratios are used. The accumulated acidogenic products caused the pH decrease and the increase of unionized volatile acid (UVA) . The extremely low pH and UVA inhibited the activity of methanogens and acidogens and may even lead to a failure of the entire anaerobic process (Wang et al., 1999). Many experimental efforts have aimed to alleviate this inhibition, including lowering the product concentrations (Gan et al., 2008), electrodialysis (Yi et al., 2008), pervaporation (Cui et al., 2004) and removal of the products in situ (Cheng et al., 2010). Adsorptions by porous media such as zeolite (Wang et al., 2011), activated carbon (Pyrzynska and Bystrzejewski, 2010) and resin (Lin and Juang, 2009) are an interesting method for in-situ removal. In recent years, andesite porphyry (known as wheat-rice-stone, WRS, in Asia), a kind of natural clay mineral, has been applied as a candidate to remove the acidogenic products in situ and thereby assist hydrolysis and acidogenesis (Cheng et al., 2010). WRS not only adsorbs the accumulated acidogenic products due to its unique tetrahedral structure with micro- and nano-channels, but also dissociates and releases cations, including Ca^{2+}, Mg^{2+} and Na^{+}, during the acidogenesis of solid food wastes (Li et al., 2009; Cheng et al., 2010). The dissociated cations provide nutrition required by the microbes (Cheng et al., 2010). The acidogenic product adsorption and cation dissociation contribute positively to the anaerobic fermentation of solid food wastes.

2.2. Objectives

In this study, the rotational drum fermentation (RDF) system with methanogenic leachate recirculation has been developed. The objectives of the work were to (1) develop a simultaneous pretreatment and acidogenesis (SPA) systems for the food wastes as part of anaerobic fermentation by an RDF system with methanogenic leachate recirculation and (2) enhance pretreatment and acidogenesis of solid food wastes by methanogenic leachate recirculation and WRS addition.

2.3. Materials and methods

2.3.1. Materials

(1) Substrate and seeding sludge

Fresh soybean meal (approximately 24.1% total solids, TS) was collected from a dining hall to be used as the substrate. The composition of the dry soybean meal was as follows: protein (22.6%), lipid (19.6%), sugar (37.0%), cellulose (14.5%), ash (6.1%) and other constituents (0.2%). The initial mean particle size of the raw material was 673 μm.

Anaerobic digestion sludge was taken from a municipal wastewater treatment plant for use as the seeding sludge. The TS, VS and of the sludge were 2.6%, 1.4% and 7.8, respectively. The initial volatile fatty acids of the substrate and the sludge were 0 and 0.04 g L^{-1}, respectively.

(2) Andesite porphyry

Andesite porphyry (WRS) was collected from a mine for use as an additive during the hydrolysis and acidogenesis of the solid food wastes. The chemical composition of the WRS used was as in Li et al (Li et al., 2009). The WRS was washed 2-3 times with distilled water and then dried in an oven at 105 ℃ to constant weight.

(3) Methanogenic leachate

The methanogenic leachate was obtained from a sound mesophilic (35-37 ℃) methanogenic process. The methanogenic process was fed daily with acetic acid and synthetic wastewater (Chang et al., 1982) and run well in long term. The methanogenic effluent was centrifuged at 3000 rpm for 3 min, and the supernatant was recycled into the acidogenic process as the methanogenic leachate. The average pH of the leachate was 7.2.

2.3.2. Experimental apparatus

The RDF system developed by Jiang et al (Jiang et al., 2005) was employed to perform the simultaneous pretreatment and acidogenesis of the solid food wastes (shown in Figure 2-1 a and b). The RDF system consisted of six drum fermentors, and each fermentor's working volume was 3.6 L. The mechanical pretreatment was mainly performed by rotating the fermentor with 26 aluminum oxide milling balls (diameter=30 mm), taking up 10% of each fermentor (in volume). Each fermentor

was rotated automatically for 15 min every 45 min at 12 rpm and 35 ± 1 ℃ during the experimental period.

2.3.3. Experimental procedure

(1) Batch operation

In batch operation, two fermentors, LB1 and LB2, were used to evaluate the effect of leachate recirculation on the simultaneous pretreatment and acidogenesis of solid food wastes, while the other four fermentors, B11, B12, B21 and B22, were used to evaluate the effect of leachate recirculation with the addition of WRS. The seeding sludge was considered as the methanogenic leachate in the batch operation. The leachate recirculation ratio (the weight ratio of methanogenic leachate to substrate) was 1:1 for LB1, B11 and B12 and 2:1 for LB2, B21 and B22. Five percent WRS (the weight ratio of WRS to substrate solid content) was added to B11 and B21, and 10% to B12 and B22. The detailed feeding conditions were shown in Table 2-1.

The batch operations lasted for 10 days. Samples were withdrawn for the analysis of pH, TS (total solids), TDS (total dissolved solids), VS (volatile solids), TVA (total volatile acids), volatile acids (VA) spectra, mean diameter (MD) and ATP concentration on alternate days.

(2) Continuous operation

Fermentors LC1, LC2, C11, C12, C21 and C22 were used for continuous

operation. Their detailed feeding conditions are shown in Table 2-2.

The hydraulic retention time (HRT) of the continuous operation was 10 days. The continuous operation was maintained with daily feedings and withdrawals for at least 3 HRTs before reaching pseudo-steady state. The pH was tested every day, while other parameters were measured on alternate days during the pseudo-steady state as for the batch operation.

2.3.4. Measurements and analyses

The pH, TS, TDS, VS and VA levels were measured using the sewage test procedure (APHA, 2005). MDs were measured using a laser particle size analyzer (LS230, COULTER). ATP concentrations, used to quantify the microbe populations, were determined using an ATP analyzer (AF-100, DKK-TOA). The sample was centrifuged at 6000 rpm for 5 min, and then the supernatant was filtered through a 0.45 μm membrane filter to assess VA spectra by a high-performance liquid chromatograph (LC-10AVP, SHIMADZU) with an Atlantic dC column (18.5μm, 4.6×150 mm, WATERS) at 30 ℃.

2.3.5. Parameter calculations for the anaerobic degradation

(1) Hydrolysis rate constant

The hydrolysis of a solid substrate can be represented by the surface based kinetics (SBK) model (Sanders et al, 2000). The hydrolysis rate constant can be

expressed as follows:

$$K_{sbk} = \rho \frac{R_0 - R_t}{t} \quad (2\text{-}1)$$

where ρ (kg m^{-3}) is the density of the substrate, R_0 (m) is the mean size of the substrate particle at time 0, and R_t (m) is the mean size of the substrate particle at time t.

(2) Total dissolved solids generated (TDS_G)

The TDS_G can be calculated using equation (2-2)

$$TDS_G = TDS_t - TDS_0 + VS_0 - VS_t \quad (2\text{-}2)$$

where TDS_t (g L^{-1}) and TDS_0 (g L^{-1}) are the total dissolved solids at time t and time 0, respectively and VS_0 (g L^{-1}) and VS_t (g L^{-1}) are the VS contents of the broth at time 0 and time t, respectively (Cheng et al., 2010).

(3) Unionized VA concentration

Unionized VA concentration (*UVA*, g L^{-1}) can be determined using following equation:

$$UVA = VA \frac{10^{(pKa - pH)}}{1 + 10^{(pKa - pH)}} \quad (2\text{-}3)$$

where *pKa* is the dissociation constant of the acid in water; the *pKa* of acetic acid is 4.762 at 35 ℃ (Weast, 1981).

(4) Specific growth rates of TDS_G, VA, and ATP

The specific growth rates of ATP or the other parameters can be calculated using

equation2-3:

$$\mu = \frac{1}{t} ln(X_t/X_{t-1}) \quad (2\text{-}4)$$

where μ (d^{-1}) is the specific growth rate of TDS_G, VA or ATP; X_t is the value of TDS_G, VA or ATP at time t; X_{t-1} is the value of TDS_G, VA or ATP at time t-1 and t is the sampling interval.

(5) VS degradation ratio (R_{VS})

The R_{VS} can be calculated using equation(2-5):

$$R_{vs} = 100 \frac{VS_0 - VS_t}{VS_0} \quad (2\text{-}5)$$

where R_{VS} (%) is the VS degradation ratio; VS_0 (g L^{-1}) is the initial concentration and VS_t (g L^{-1}) is the concentration of a sample taken from the fermentor at time t.

(6) Particle size distribution

The particle size distribution is characterized by the MD and the relative span (S_L) (Igathinathane et al., 2009; Resch et al., 2011). The relative span is determined using equation(2-6):

$$S_L = \frac{D_{90} - D_{10}}{D_{50}} \quad (2\text{-}6)$$

where S_L is the relative span; D_{10}, D_{50} and D_{90} are the diameters as the cumulative volumes reached 10%, 50% and 90%, respectively.

(7) VA yield

The VA yield is expressed by the equation as follow:

$$\eta = \frac{VA_t - VA_0}{VS_0 - VS_t} \quad (2\text{-}7)$$

where VA_t (g L^{-1}) and VA_0 (g L^{-1}) are the VA concentration of the sample at time t and time 0, respectively; VS_0 (g L^{-1}) is the initial concentration and VS_t (g L^{-1}) is the concentration of a sample taken from the fermentor at time t.

2.4. Results and discussion

2.4.1. Effect of leachate recirculation on SPA

(1) Batch operation

The PSDs of broths in LB1 and LB2 are shown in Fig.1 (a and b). The curve of the particle size distribution changed from one dominated by large particles to one dominated by smaller ones (from right to left in the figure) over the operation time. The S_Ls obtained using equation(2-6) for LB1 and LB2 peaked at 2.70 and 3.16, respectively, much higher than that of the original feedstock (1.53). The increases in S_L suggest that substrate flocs were ground into finer particles. The calculated MDs of substrate particles in the batch operations are shown in Figure 2-3 (a). The MDs decreased linearly in LB1 and LB2 from 673 to 567 and 96 μm, respectively. According to the linear regression of Figure 2-3 and equation2-1, the calculated K_{sbk} values for LB1 and LB2 were 11.4 and 50.0 $\times 10^{-3}$ kg m^{-2} d^{-1}, respectively. The K_{sbk} of LB2 was 4.4-fold higher than that of LB1. The dramatically higher K_{sbk} of LB2 was ascribed to the higher humidity that resulted from the increased leachate

recirculation ratio. The higher humidity reinforced the impact of the milling balls onto the substrate flocs, the dispersion of flocs and the shear flow in the rheology (Izumi et al., 2010). Both the higher impact and higher shear flow enhanced splitting of the substrate agglomerates and broke crystal structures.

Enrichment with the enzymes and microbes in the leachate caused biodegradation occur during the mechanical pretreatment. The VS degradation rate was determined using a regression Figure 2-4 (a). The VS degradation rates for LB1 and LB2 were 6.2 and 4.5 g L^{-1} d^{-1}, respectively. The TDS_G grew logarithmically during the batch operation. The specific growth rate of the TDS_G (μ_{TDSG}) was calculated using equation(2-4) as 0.16 and 0.17 d^{-1} for LB1 and LB2, respectively. The higher TDS_G and the VS degradation rate in LB1 were caused by the lower leachate recirculation ratio. Similar results were obtained by Zhou et al (Zhou et al., 2011). In the lower leachate recirculation ratio fermentor (LC1), the presence of more readily biodegradable substrate (the initial total dissolved solids TDS_0, 14.0 g L^{-1}) ensured the anaerobes' growth during the initial period.

The specific growth rates of the anaerobes (represented by the ATP concentration μ_{ATP} (APHA, 2005) in LB1 and LB2 were 5.40 and 5.04 d^{-1}, respectively. The growth of the anaerobes not only accelerated VS degradation, but also enhanced the formation of VAs. The specific growth rates (μ_{VA}s) for the TVAs in LB1 and LB2 were similar, with values of 0.29 and 0.28 d^{-1}, respectively. The yields of VA and ionized VA (IVA) for LB1 and LB2 tended similar to the μ_{VA}s. The VA yields for LB1

and LB2 were 0.16 and 0.15 g-VA/g-VS, respectively. The IVA ratio of LB2 was higher than that of LB1 during the batch operation, despite the finding that the IVAs for both LB1 and LB2 were no more than 30% of the TVAs. Apparently, more leachate recirculation had a greater effect on the mechanical than the biochemical aspect of the in this work.

(2) Continuous operation

The parameters in the continuous operation trials were obtained by averaging the data obtained under steady state. The particle size distributions of LC1 and LC2 are shown in Figure 2-2. The particles spanned broader ranges in LC1 and LC2 than in the feedstock. The S_Ls of LC1 and LC2 were higher by 31.9% and 35.2% than that of the feedstock (1.53), respectively. The higher S_L of LC2 indicated that substrates were ground into finer particles in this fermentor. The pretreatment characteristics of the continuous operations are summarized in Table 2-3. The MDs for LC1 and LC2 were reduced from 744 μm in the feedstock to 662 and 491 μm, respectively. The calculated K_{sbk} of LC2 was higher by 2.1-fold than that of LC1. Higher K_{sbk} and lower MD were obtained at the higher moisture content (LC2), which coincided with the results in the batch operation. The substrate particles were stressed mechanically by the compression, impact and the friction of the milling balls in this work. The breakage of substrate particles depended on the yield stress of itself and the stressing intensity. The yield stress dropped at certain higher moisture content (Müller et al., 2013), meaning particles were apt to be broken mechanically. In addition, the particles

were softened in the structure, thus the accessibility of the substrate was enhanced. This was evidenced by the results and R_{VS} and TDS_G.

The R_{VS} of LC2 was 2.4-fold higher than that of LC1. Similar to the VS degradation, the TDS_G for LC2 was more than 2-fold greater than that of LC1. The finer particles presented larger surface area, and available for the microorganisms, resulting a higher R_{VS} and TDS_G. The solubility of substrate were also enhanced by the wet ball milling pretreatment (Izumi et al., 2010). In another word, these favorable TDS_Gs were generated by the combination of mechanical pretreatment with biochemical reactions. As shown in Table 2-4, the apparent VA yield of LC1 was 2.5-fold above that of LC2 despite their similar TVAs. The IVAs predominated in both LC1 and LC2. The IVA values were elevated significantly compared to those under continuous operation without leachate recirculation (Chen et al., 2008). The higher IVAs favored higher rates of methanogenesis.

The VA spectra appeared to be influenced by the leachate recirculation. The acetic acid contents of LC1 and LC2 were 31.1% and 14.6%, respectively, while the propionic acid contents were 20.7% and 36.5%, respectively. The ratio of propionic acid to acetic acid (P/A) of LC2 greater than 1.4 or the was possibly considered as an indicator of failure (Hill et al., 1987), however, the lower amount of acetic acid in LC2 was caused by its conversion to biogas (methane and carbon dioxide) in this work. The biogas emission was observed during daily operations, during which 70% methane was generated from acetic acid. On the contrary, little biogas was observed

in LC1. The rich leachate in LC2 introduced more microbes and enzymes and favored degradation of the acetate.

2.4.2. Effects of leachate recirculation and WRS addition on SPA

(1) Batch operation

Time courses for particle size distributions during the batch operations are shown in Figure 2-2 (c-f). The calculated S_Ls for B11, B12, B21 and B22 were 3.0, 2.49, 3.44 and 2.98, respectively. The S_L for each run increased over the course of the reaction. The time courses for MDs during batch operation are shown Figure 2-3 (b). The MDs for B11, B12, B21 and B22 were 494, 294, 158 and 61 μm, respectively. The MDs for B11 and B12 were lower by 12.8% and 48.1% than that of LB1, respectively. The MD for B21 was higher than that of LB2, while the MD for B22 was 63.5% of LB2's. Correspondingly, the K_{sbk}s obtained for B11 and B12 were 16.0 and 29.8 $\times 10^{-3}$ kg m^{-2} d^{-1}, and 50.0 and 65.0$\times 10^{-3}$ kg m^{-2} d^{-1} for B21 and B22, respectively. The K_{sbk}s for B11 and B12 were 1.4 and 2.6-fold higher than that of LB1 respectively. The K_{sbk} for B21 was close to that of LB2 (50.0 10^{-3} kg m^{-2} d^{-1}), while the K_{sbk} for B22 was 1.3-fold higher than that of LB2. Compared to LB1 and LB2, the WRS addition significantly enhanced the substrate particle size reduction (except B21). Moreover, the WRS addition led to a faster substrate particle size reduction in the case of the higher leachate recirculation ratio. The WRS particles were much harder than the substrate particles. The elasticities of the WRS and substrate mixtures

were lower than that of the substrate alone. With WRS addition, the substrate particles were more easily broken when they were impacted by the milling balls. In addition, the large amounts of cations such as Ca^{2+}, Na^{+} and Al^{3+} in the WRS are prone to dissociation and formation of compounds such as $CaCl_2$ and CH_3COONa in acidic broth. In the experiment, the compounds may have arranged their dipoles to reduce the flocs' surface energies and thereby aided the fragmentation of the solid substrates.

The time course of VS degradation and TDS_G is shown in Figure 2-4. The VS degradation rates for B11, B12, B21 and B22 were 7.0, 6.5, 4.6 and 5.5 g L^{-1} d^{-1}, respectively. The VS degradation rates for B11 and B12 were higher by 11.7% and 4.8% than that of LB1, respectively. The VS degradation rate for B21 was similar to that of LB2, while that of B22 was higher by 22.2% than that of LB2. The TDS_G grew logarithmically during the batch operation. The μ_{TDSG}s for B11, B12, B21 and B22 were 0.15, 0.09, 0.07 and 0.13 d^{-1}, respectively. Higher leachate recirculation and more WRS addition were therefore favorable to the VS degradation.

The apparent VA yields for B11, B12, B21 and B22 were 0.09, 0.26, 0.16 and 0.31 g-VA/g-VS, respectively. Compared to LB1 and LB2, more WRS addition favored more VA production (Li et al., 2009; Cheng et al., 2010). Formic acid predominated in fermentor B11 and B21, while succinic acid did in fermentor B12 and B22 during the initial period. Acetic acid predominated beginning on the 4^{th} day in all the fermentors. The specific growth rates for acetic acid in B11, B12, B21 and B22 were 0.31, 0.30, 0.24 and 0.31 d^{-1}, respectively. The occupation of acetic acid was elevated by the

WRS addition compared with LB1 and LB2. These results suggest that the WRS addition promoted the conversion of long-chain VAs to short ones. Among the short chain VAs, propionic acid is difficult to be converted to acetic acid by the microorganisms compared to butyric acid. On the contrary, acetic acid was prone to consumed by the methanogens. The higher acetic acid occupation implied a potential for the biogas production.

The data for VS degradation and VA production demonstrate that the WRS addition enhanced the biochemical reactions. These results were consistent with the anaerobes' growth as the μ_{ATP}s for B11, B12, B21 and B22 increased by 5.9%, 24.5%, 21.0% and 2.4% compared to LB1 and LB2.

(2) Continuous operations

The parameters of the continuous operations were obtained by averaging data obtained under steady state. The particle size distributions are shown in Figure 2-2. Substrate particles smaller than 100 μm were 5.7%, 11.7%, 16.4% and 32.6% of the totals, respectively, in comparison to those of LC1 (6.2%) and LC2 (8.7%). Aldin's work (Aldin, 2010) suggested that most biodegradable matter ranged from 0.001-100 μm. In our work, the WRS addition contributed to the conversion from raw materials to materials with high proportions of biodegradable constituents. The S_Ls calculated using equation(2-6) for C11, C12, C21 and C22 were 1.99, 2.36, 2.64 and 3.02, respectively. The S_L of C11 was slightly lower than that of LC1, while the S_L of C12 was higher by 16.8% than that of LC1. The S_Ls of C21 and C22 were higher by 27.5%

and 45.9% than that of LC2, respectively. These results suggest that more fine particles were produced due to the WRS addition.

Characteristics of the pretreatments are shown in Table 2-3. The MDs of fermentors C11, C12, C21 and C22 decreased from 673 μm to 656, 628, 518 and 351 μm, respectively. The MDs of C11 and C12 were slightly lower than that of LC1. The MD of C21 was slightly higher than that of LC1, while the MD of C22 was lower by 39.8% than that of LC2. Correspondingly, the K_{sbk}s for C11 and C12 were higher by 7.3% and 41.5% than that of LC1, respectively. The K_{sbk} for C21 was close to that of LC2, while the K_{sbk} for C22 was higher by 54.7% than that of LC2. The higher leachate recirculation and WRS addition ratio led to the substrate particle shift from the large to the micro range.

The R_{VS}s and the TDS_Gs are shown in Figure 2-4. The R_{VS}s of C11 and C12 were close to that of LC1, whereas those of C21 and C22 were much higher than that of LC2. The R_{VS}s of C21 and C22 were higher by 35.8% and 63.9% than that of LC2, respectively. The WRS was prone to dissociate cations, including Fe^{2+}/Fe^{3+}, Ca^{2+} and Mg^{2+}, and accelerated the oxidation/reduction reaction under the high leachate recirculation ratio. On the contrary, the WRS dissociation and cation transfers were perturbed in case of the lower leachate recirculation ratio. The leachate recirculation contributed 64.8% and 64.5% to the VS degradation, while WRS addition contributed 35.2% and 35.5% to that in C11 and C12, respectively. The values for TDS_G showed similar trends to those for the VS degradation ratio. The highest TDS_G achieved was

in C22, which was higher by 56.2% than that of LC2.

The TVA of C11 was higher by 8.7% than that of LC1, while the TVA of C12 was lower by 2.9% than that of LC1. The VA formation seemed to be little enhanced by WRS addition in the case of the lower leachate recirculation ratio, even though the TVAs for C21 and C22 were supposed to increase. Interestingly, the TVAs of C21 and C22 were lower by 14.4% and 28.8% than that of LC2, respectively. The decreased VAs were converted to biogas. In our work, biogas emission was observed in C21 and C22 during daily operations. The biogas emission should not only be ascribed to the reduction of particle size (Zhang and Banks, 2013), but also resulted from the WRS addition. As mentioned above, the cations dissociated from WRS had the enzyme and microorganisms sparked and promoted the acidogenesis and even the methanogenesis potentially.

Similar results were obtained for the distributions of the IVAs and UVAs and for the VA spectra. The IVAs predominated in all the fermentors, which was possibly conducive to biogas formation. Compared to LC2, the acetic acid occupation decreased with the increased addition of WRS in C22. The decreased acetic acid was possibly converted to biogas. Notably, the occupation of propionic acid varied in the range of 29.2-58.5%. The TDSs were prone to conversion to propionate rather than to other VAs (such as butyrate, acetate and lactate) by lower energy demand during the biochemical reaction. However, propionate is more difficult to convert to acetate than to butyrate and lactate (Azbar et al., 2001).

2.5. Conclusion

In this section, a simultaneous pretreatment and acidogenesis intended for the solid food waste has been developed by both batch and continuous operations. The methanogenic leachate was recycled into the RDF and considerably improved the pretreatment of the recalcitrant contents in the solid food waste. Effects of leachate recirculation ratio were investigated and results demonstrated that a higher leachate recirculation ratio from the methanogenesis to RDF system boosted the mechanical pretreatment, such as particle size reduction and TDS generation in anaerobic process. The methanogenic recirculation ratio of 2 was considered as a better condition for the anaerobic fermentation of solid food waste

Based on the elucidation of leachate recirculation at various ratios, effects of WRS addition on the pretreatment and acidogenesis of the solid were studied by batch and continuous operation as well. Compared the control (without WRS), the 10% WRS addition at higher leachate recirculation ratio obviously enhanced the VS degradation, particle size reduction, VA formation and the subsequent progress of biogas conversion.

Table 2-1 Operation conditions for the batch operation experiments

Fermentor	Substrate and methanogenic leachate			WRS addition	
	Weight of methanogenic leachate (g)	Weight of Soybean meal (g)	Weight ratio of methanogenic leachate to substrate	Weight (g)	Proportion (in substrate TS, %)
LB1	1200	1200	1:1	0	0
LB2	1600	800	2:1	0	0
B11	1200	1200	1:1	12	5
B12	1200	1200	1:1	24	10
B21	1600	800	2:1	8	5
B22	1600	800	2:1	16	10

Table 2-2 Daily operation conditions for the continuous operation experiments

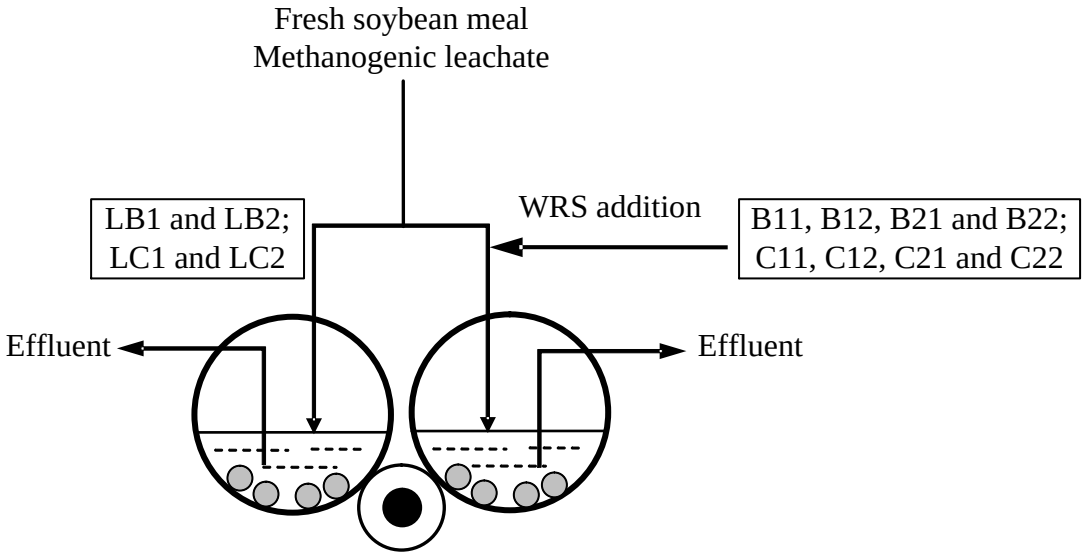
Fermentor	Substrate and methanogenic leachate			WRS addition	
	Weight of methanogenic leachate (g)	Weight of soybean meal (g)	Weight ratio of methanogenic leachate to substrate	Weight (g)	Proportion (in substrate TS, %)
LC1	90	90	1:1	0	0
LC2	120	60	2:1	0	0
C11	90	90	1:1	0.9	5
C12	120	120	1:1	2.4	10
C21	120	60	2:1	0.6	5
C22	140	70	2:1	1.4	10

Table 2-3 Pretreatment characteristics of the continuous operations under steady state

Fermenter No.	PSD (μm)	K_{sbk} (10^{-3} kg m^{-2} d^{-1})	R_{VS} (%)	TDS_G (g L^{-1})	S_L -
LC1	662 ±12	8.2	17.1	20.8 ±0.2	2.02 ±0.01
LC2	491 ±11	25.4	41.6	42.9 ±0.1	2.07 ±0.02
C11	656 ±9	8.8	11.1	13.6 ± 0.0	1.99 ±0.02
C12	628 ±15	11.6	16.2	21.4 ± 0.1	2.36 ±0.03
C21	518 ±6	22.6	56.5	45.5 ± 0.0	2.64 ±0.03
C22	351 ±15	39.3	68.2	67.0 ± 0.0	3.02 ±0.03

Table 2-4 Acidogenesis characteristics of the continuous operations under steady state

Fermenter No.	pH(-)		VA	Apparent Y_{VA}	VA distribution		VA spectra (%)			
	In	Out	(g L^{-1})	g-VA g^{-1}-VS	UVA (%)	IVA (%)	Acetic acid	Propionic acid	Butyric acid	Succinic acid
LC1	7.5	5.2	10.9	0.58	26.7	73.3	31.1	20.7	24.6	23.6
LC2	7.5	5.0	10.4	0.23	36.6	63.4	14.6	36.5	26.4	22.5
C11	7.4	5.1	11.7	0.97	31.5	68.5	-	-	-	-
C12	7.5	5.1	10.1	0.73	31.5	68.5	37.9	29.2	25.1	7.8
C21	7.4	5.0	8.9	0.21	36.6	63.4	17.3	58.5	23.1	1.1
C22	7.5	4.9	7.4	0.12	42.1	57.9	9.0	29.3	27.1	34.6



(a)

(b)

Figure 2-1 Apparatus for the simultaneous pretreatment and acidogenesis system for the solid food waste

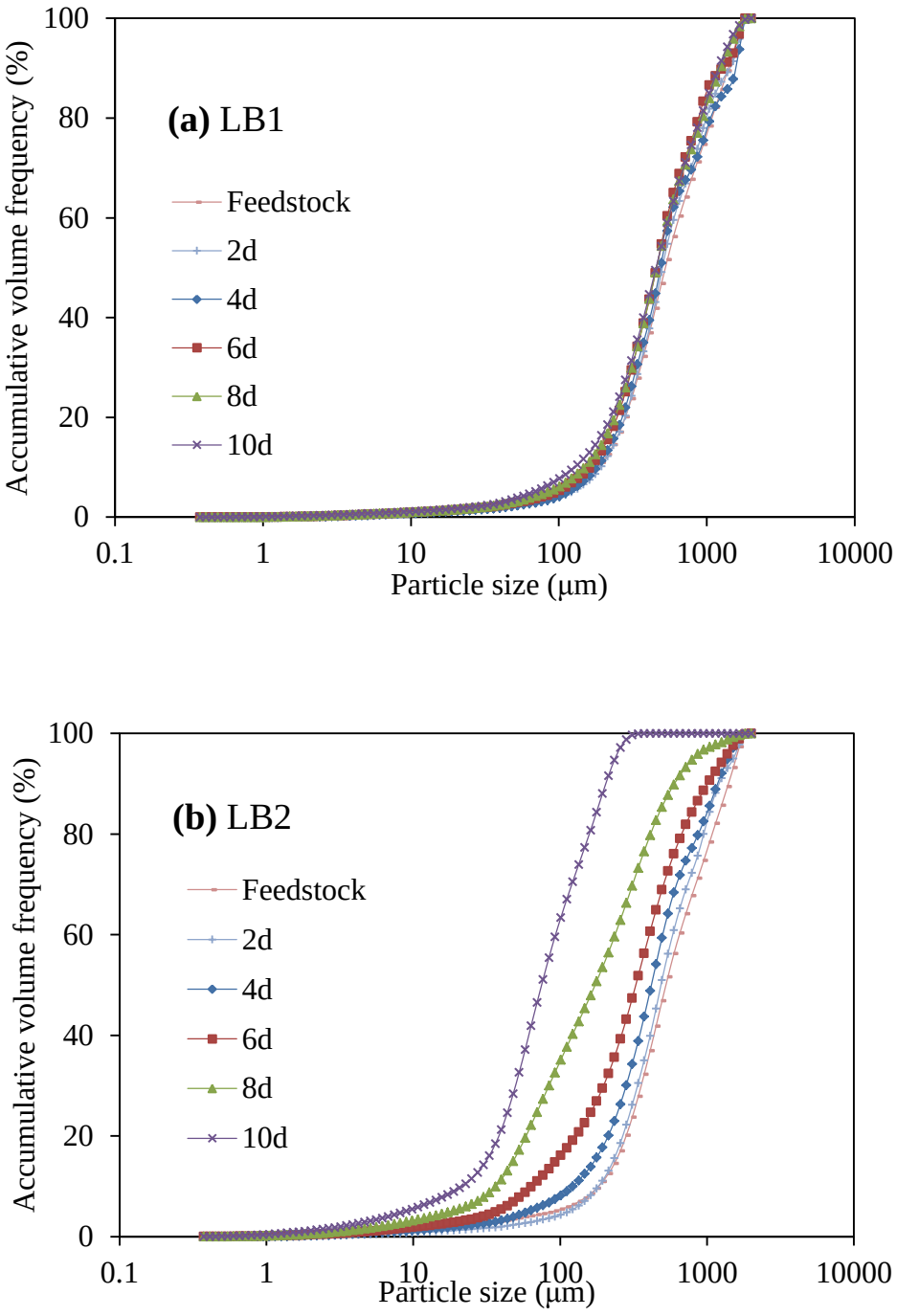
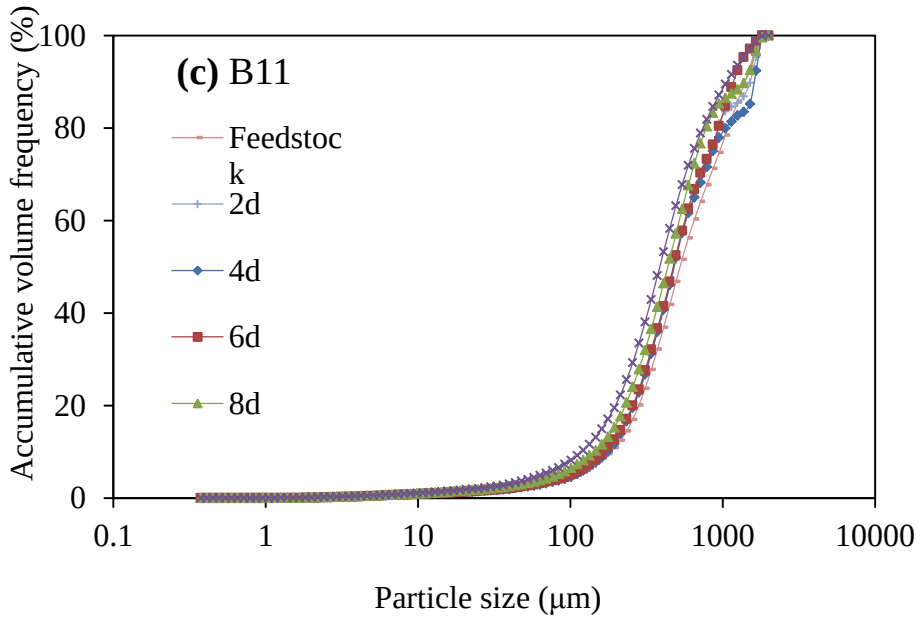


Figure 2-2 Particle size distributions in the batch operations



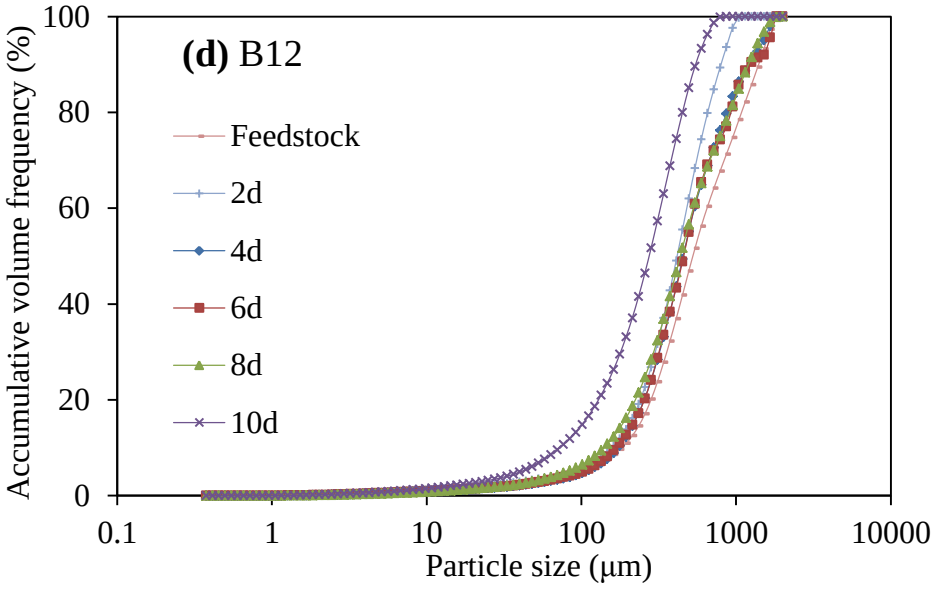


Figure 2-2 Particle size distributions in the batch operations

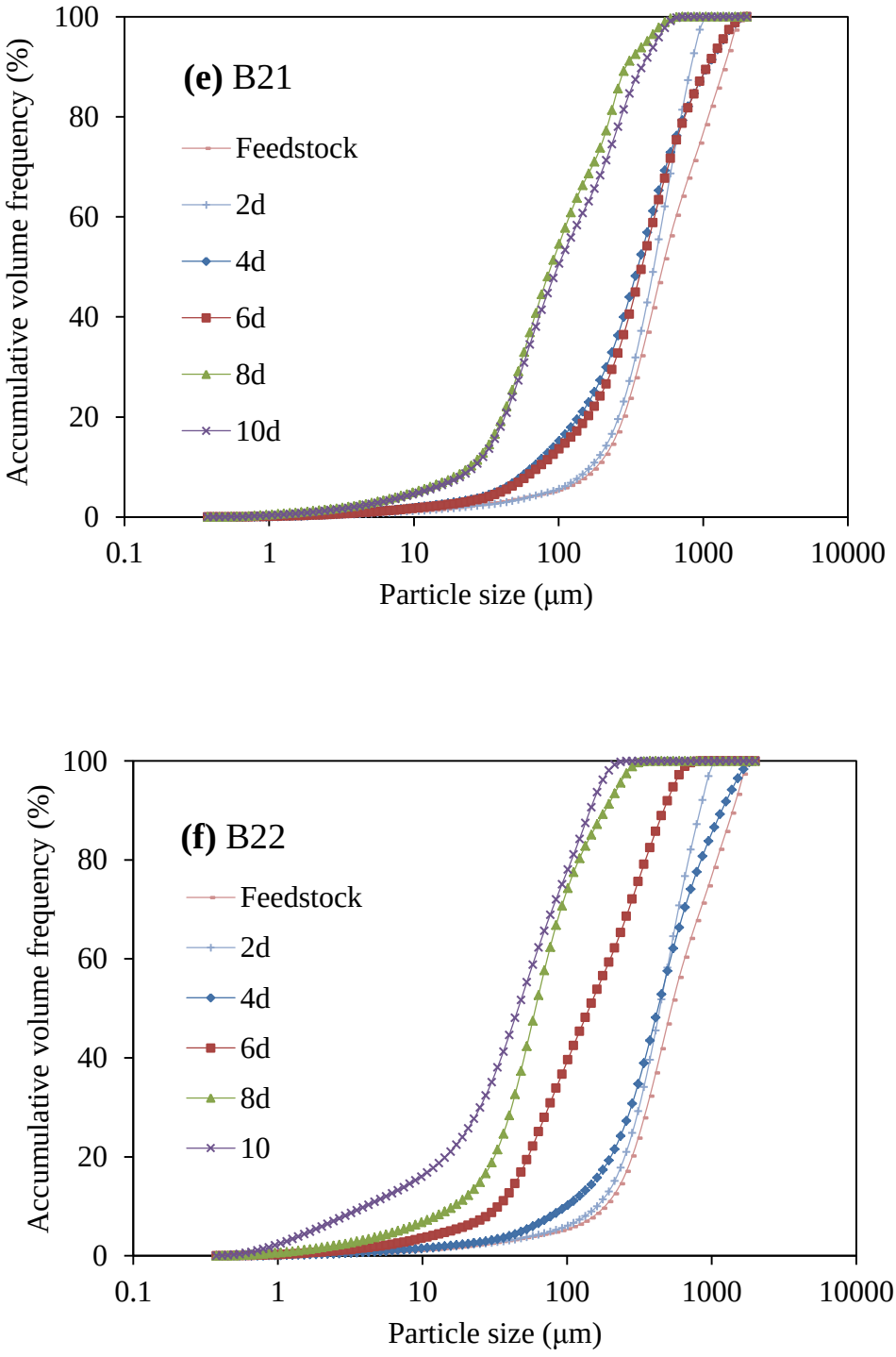


Figure 2-2 Particle size distributions in the batch operations

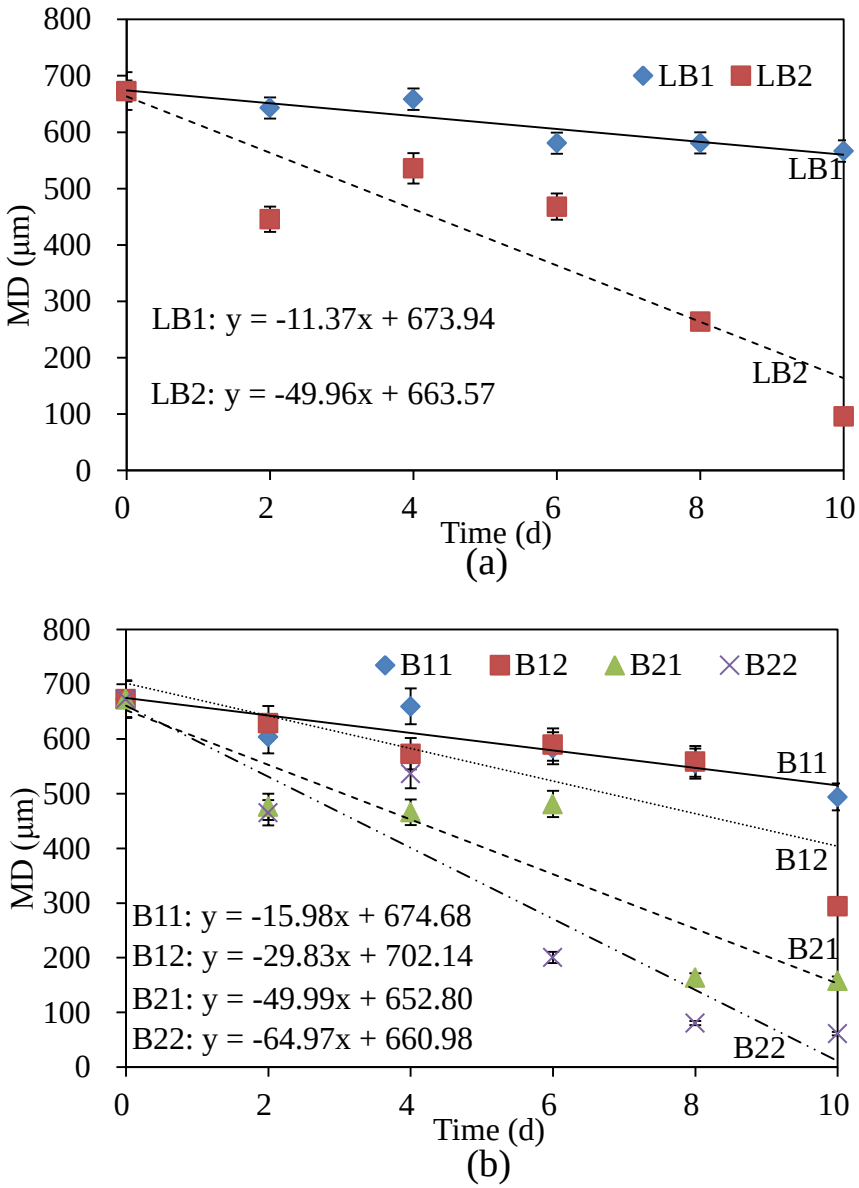


Figure 2-3 Time courses for particle MDs in the batch experiments

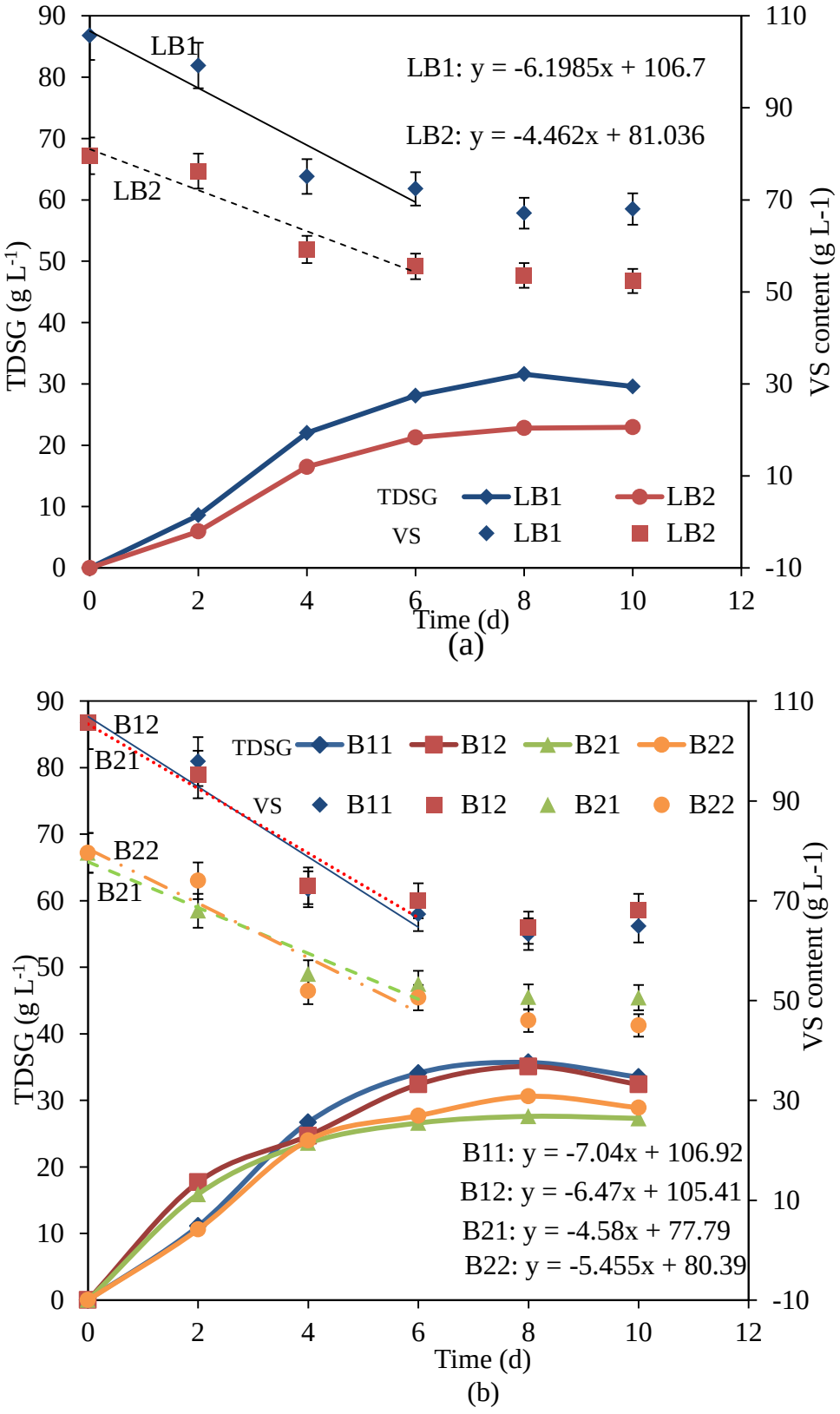


Figure 2-4 Time courses for TDS_G and VS contents

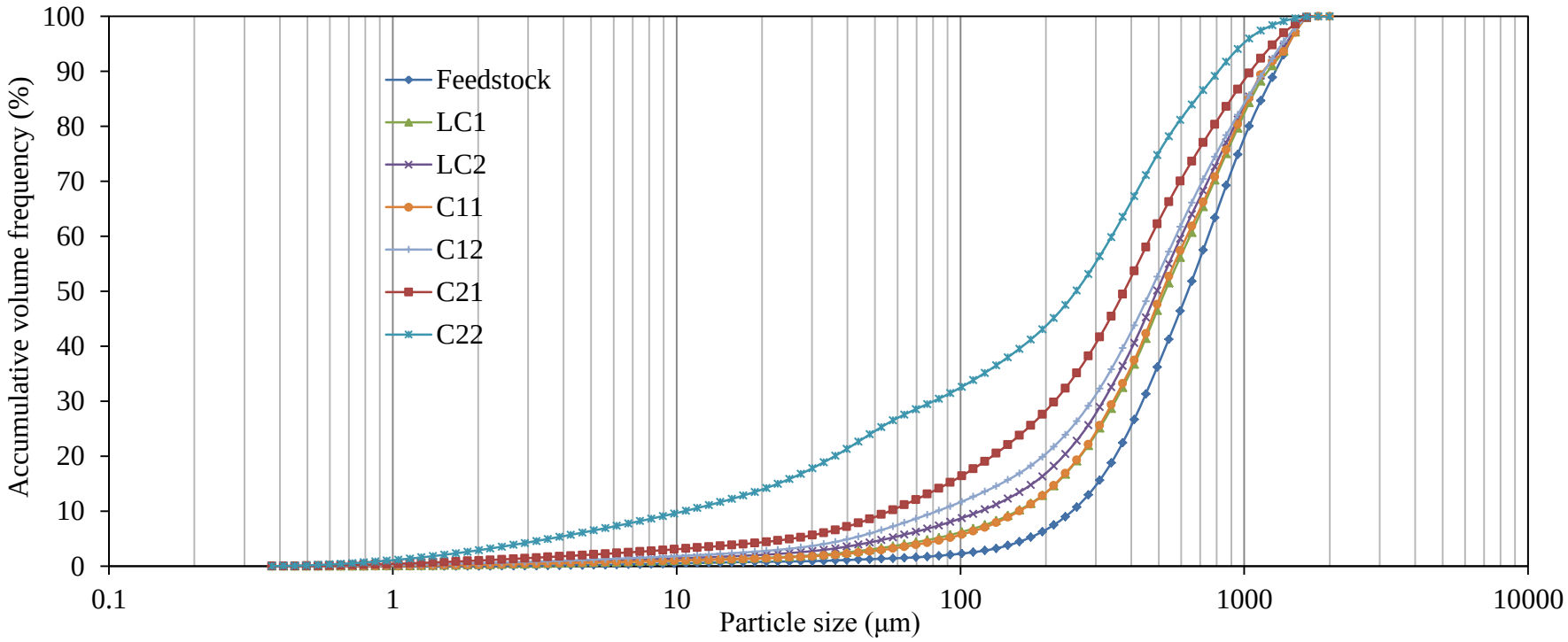


Figure 2-5 Particle size distributions in the continuous operations

Chapter 3

Characteristics of an alkali-free system for liquid waste from food processing

3.1. Introduction

Ammonia as a renewable bio-energy candidate has attracted a great deal of research recently from various companies. Ammonia is a carbon neutral substance, which is understood to be a good storage medium for hydrogen (Lan et al., 2012). Current hydrogen liquefaction storage options risk the leakage of hydrogen and resulting security problems due to the demands of extremely high pressure. By contrast, ammonia will liquefy at -33 °C under ordinary pressures. Therefore, ammonia boasts a 50% higher specific energy density (kW h L^{-1}) than that of liquefied hydrogen (Vitse et al., 2005). Moreover, ammonia itself is also a type of efficient bio-energy compound. The energy density of ammonia is comparable with coal and petroleum (Klerke et al., 2008; Zuttel et al., 2010). Research into ammonia fuel cells has been conducted in the most recent decade. Ammonia has been proposed for use in polymer electrolyte membrane (PEM) fuel cells (Chellappa et al., 2002). A

more flexible scale of operation is allowed through investigations into ammonia solid-oxide fuel cells (Ma et al., 2006; Zhang and Yang, 2008).

Ammonia is the second most widely produced commodity chemical in the world, and over 100 Mt is transported every year Leggett and Ball, 2012(Jensen et al., 2007; Cheddie, 2012). Over 80% ammonia is produced by a synthetic process using fossil fuels, mainly natural gas and coal. Ammonia synthesis not only consumes a great deal of fossil fuels and intensifies the fuel crisis, but also increases greenhouse gas emissions. As an alternative, ammonia could be recovered by bio-chemical conversion from protein-rich waste/wastewater during or prior to the anaerobic fermentation process (Walker et al., 2011; Yabu et al., 2011). On the contrary, ammonia is always considered as the the inhibitor for methane production rather than a useful biomass resource in most researches. Many methods were taken to remove ammonia/ammonium, such as dilute the digestate, adsorption, struvite precipitation and stripping with the alkali addition (Senturk et al., 2010; Yabu et al., 2011). Stripping has been paid more attention because it could recover ammonia as a resource of fertilizer. Stripping used in most researches and engineering consumes a great deal of alkali to adjust the pH to 8-10. The introduction of high strength cation challenges the microbes' tolerance and may deteriorate the whole anaerobic process. Therefore, a system oriented to alkali-free bioammonia production and biomass

recovery is necessary.

One of critical factors for ammonia recovery is the ammonium concentration in the anaerobic broth. According to the chemical equilibrium, high concentration ammonium favours the ammonia production and volatilization. Ammonium generated from the hydrolysis of proteinaceous organic substrates has been been considered as a toxic for anaerobic fermentation under both thermophilic and mesophilic conditions. Most relevant studies have been conducted to kinetically evaluate the methane production in case of ammonia/ammonium (Vavilin et al., 1994; Vavilin et al., 1995). By comparison, little work concentrated on the simulation of bioammonium production. For example, mathematic models (Angelidaki et al., 1993) were used to demonstrate the interactions among ammonia, pH and temperature in anaerobic fermentation of manure, but no model was developed to predict ammonia/ammonium concentration during the anaerobic process. The first-order rate model has been used to evaluate the methane production by anaerobic process involving ammonia/ammonium inhibition or stripping (Walker et al., 2011), but no information was provided for the kinetic analysis of ammonium production. From this perspective, the kinetic analysis of ammonium production by anaerobic fermentation is in need.

3.2. Objectives

There are two objectives for this work: (1) develop an alkali-free system to produce ammonia/ammonium by anaerobic fermentation of protein rich wastewater and (2) establish a kinetic model for bioammonium production to predict ammonium concentration and attain the optimal conditions for bioammonium production in anaerobic fermentation.

3.3. Materials and methods

3.3.1. Substrate and seeding sludge

The seeding sludge was collected from a sewage treatment centre in Ibaraki, Japan. The pH, total ammonium nitrogen (TAN), total suspended solids (TSS) and volatile suspended solids (VSS) were 7.5, 434 mg L^{-1}, 2.1% and 1.1%, respectively.

A soluble protein powder (ORIHIRO, Tokyo) was used to prepare the synthetic wastewater as the substrate. The powder is made from soy protein. The composition of the powder is as follows: protein, 81.2%; lipid, 0.1-5%; carbohydrate, 2-12%; Na, 0.43-1.2%; K, 0.25%; Ca, 0.80%; Mg, 0.04-0.11%; Fe, 0.005-0.015%; Vitamin B1, 0.016%; and Vitamin B2, 0.018%. Co (0.05 mg L^{-1}) and Ni (0.5 mg L^{-1}) were added to the synthetic wastewater as trace elements. The ratio of carbon-to-nitrogen in the

synthetic wastewater was 3.9.

3.3.2. Experiment apparatus

The apparatus for ammonia production is presented in Figure 3-1. The height of the fixed bed reactor was 50 cm with a height-to-diameter ratio of 5:1. The working volume for the reactor is 2.6 L. Pall rings, which are made of plastic and have diameters of 25 mm, were packed into the reactor.

The wastewater was fed from the bottom of the reactor and sampled at a middle port with a magnetic valve controlled by a timer. The biogas produced from the system went through an ammonia trap (2 M sulphuric acid) before being stored in the gas collector. The biogas was recycled through the system to mix the broth and strip out the free ammonia.

3.3.3. Experimental procedure

(1) Sludge cultivation

The sludge was cultivated by the nitrogen-rich synthetic wastewater for a hydraulic residence time (HRT) of 4 days. The pH, TAN, TSS and VSS of the cultivated sludge were 6.9, 997 mg L^{-1}, 1.0% and 0.6%, respectively. The cultivated sludge was used as the seeding sludge in this work.

(2) Effect of substrate concentration on ammonia production by anaerobic fermentation

Semi-continuous operation was used to investigate the effect of substrate concentration on ammonia production by anaerobic fermentation. Four reactors labelled FSN1, FSN2, FSN3 and FSN4 were employed in the experiment. The substrates for FSN1, FSN2, FSN3 and FSN4 contained 2340, 2740, 3140 and 3540 mg-N L^{-1}, respectively. The semi-continuous was operated at 37 ±1℃ for an HRT of 4 days.

(3) Effect of HRT on the ammonia production by anaerobic fermentation

Two reactors labelled H2 and H4 were employed in this semi-continuous experiment. An experiment with an HRT of 4 d was performed in reactor H4 while successive experiments with HRTs of 2, 1 and 0.5 d were performed in reactor H2. For reactor H2, the experiments over HRTs of 2, 1 and 0.5 d were carried out during the 0-10^{th} day, 10-15^{th} day, and 15-18.25^{th} day, respectively. Each substrate's total nitrogen concentration (TN) was determined by performing the experiment discussed in subsection 2.3.2. The experimental conditions were the same as in the experiments where the effect of substrate concentration on ammonia production was studied.

3.3.4. Analytical methods

The pH and TAN were monitored daily. TSS, VSS, total volatile acid concentration (TVA) and TN were measured during steady state.

The pH was tested with a pH meter (TRX-90, TOKO), and TAN was tested using an ammonia electrode (Ti-9000, TOKO). TSS and VSS tests followed the Standard Methods for the Examination of Water & Wastewater (APHA, 2005). TVA and VA spectra were tested by a gas chromatograph (Shimadzu GC-14B) with a column (Unisole F-200) containing carrier gas (N_2) flow of 40 mL min^{-1}. TN was detected by a TOC/TN analyser (TOC-V_{CPH} Shimadzu) with a nitrogen analyser unit (TNM-1).

3.4. Results and discussion

3.4.1. Effect of substrate concentration on ammonia production

(1) Characteristics of ammonia production at various substrate concentrations

The time course of pH and TAN for various substrate concentrations are shown in Figure 3-1. TAN strongly increased from the initial value of 997 mg L^{-1} due to degradation of the protein before gradually reaching a steady state. TAN was released from the enzymatic processing of proteins. The average values of TAN at the steady state were 1722, 1862, 2123 and 1976 mg L^{-1} for FSN1, FSN2, FSN3 and FSN4,

respectively (as shown in Table 3-1). To a certain extent, steady-state TAN values increased with the increase of the substrate concentration. Compared to FSN1, steady-state TAN values in FSN2, FSN3 and FSN4 were higher by 8.1%, 23.3% and 14.8%, respectively. The pattern-breaking decrease of steady-state TAN value in FSN4 was caused by the high substrate concentration. The excessive substrate concentration produced an overloaded feedstock, which led to an inhibition of the protein degradation and, consequently, ammonia production.

In anaerobic fermentation, the protein is first hydrolysed by proteolytic enzymes into peptides and amino acids (Hoover and Stokes, 1991). Peptides and amino acids are degraded to VA and ammonium prior to conversion to acetate by acetogens and methane and CO_2 by methanogens. Therefore, TVA concentration partially reflects the hydrolysis and degradation of the protein. The TVA concentrations for FSN1, FSN2, FSN3 and FSN4 were 7.2, 11.5, 12.5 and 15.4 g L^{-1}, respectively. TVA increased with the increased substrate concentration and TAN, which indicates a slow VA degradation and conversion. In particular, acetic acid concentration dramatically increased (shown in Table 3-1) and became dominant in all four runs. Acetic acid concentrations grew from 2.9 to 6.6 g L^{-1} while the substrate concentrations and TAN increased from 2344 to 3540 mg-N L^{-1} and 1722 to 1976 mg L^{-1}, respectively. This result suggests that methanogenesis was inhibited. Similar results were presented by

Angelidaki and Ahring (Angelidaki and Ahring, 1994). The propionic acid amount varied in the vicinity of 15-20%. Propionic acid is prone to accumulate during anaerobic fermentation and may be an inhibitor of the process. The ratio of propionic acid to acetic acid was proposed by Hill (Hill et al., 1987) as an indicator to predict the failure of anaerobic fermentation. The ratios of propionic acid to acetic acid in our experiments were 0.30, 0.40, 0.49 and 0.36 for FSN1, FSN2, FSN3 and FSN4, respectively; all values are much lower than the suggested threshold of 1.4. Noticeably, the ratio of propionic acid to acetic acid grew with an increase of the substrate's nitrogen concentration (except FSN4), which is consistent with a previous report (Marchaim and Krause, 1993).

Although TVA at steady state did vary among the 4 runs, the variations in pH seemed mild. pH at the steady state varied in the range of 6.7-6.9 among the four runs. A similar phenomenon was also reported by Senturk et al (Senturk et al., 2010). TVA in an acidic environment mainly existed in the form of molecules, and free H^+ existed in small amounts in the broth due to the VA ionisation equilibrium. In addition, pH is not strongly sensitive to variations in H^+ because it is the decimal logarithm of the reciprocal of H^+. The coexistence of NH_4^+ and HCO_3^-, which were produced simultaneously, played the role of a buffer in the broth to keep the pH stable.

As shown in Table 3-1， the VSS for FSN1, FSN2, FSN3 and FSN4 were 0.63,

0.61, 0.80 and 0.88%, respectively. VSS values in FSN3 and FSN4 were considerably higher than those of FSN1 and FSN2. Previous research demonstrated that ammonia is used by the organisms as an essential nutrient for assimilation (Hill, 1982; Angelidaki et al., 1993):

$$CH_3COOH + 0.022NH_3 = 0.022C_5H_7NO_2 + 0.945CH_4 + 0.945CO_2 + 0.066H_2O \quad (3\text{-}1)$$

The higher VSS levels implied a higher biomass concentration in FSN3 and FSN4 due to the more substantive substrates. This could also explain the reason that TAN in FSN4 decreased compared to that of FSN3: more nitrogen in FSN4 was possibly used by the biomass to proliferate. The biomass proliferation in FSN3 and FSN4 indicated good microbe metabolism and ammonia production activity.

(2) Kinetic analysis

The effect of substrate concentration was evaluated using the Michaelis-Menten (M-M) model as follows:

$$r = \frac{r_{\max} C_S}{K_m + C_S} \quad (3\text{-}2)$$

where r (mg-N·L^{-1}·d^{-1}) is the ammonium production rate, r_{max} (mg-N·L^{-1}·d^{-1}) is the maximum ammonium production rate, C_s (mg-N L^{-1}) is the substrate concentration, and K_m (mg-N L^{-1}) is the substrate concentration at which the reaction rate is half of the maximum.

The M-M equation was reformulated into a Hanes-Woolf (H-W) plot to calculate

the kinetic parameters in this work. The H-W plot is expressed as follows:

$$\frac{C_S}{r} = \frac{K_m}{r_{\max}} + \frac{C_S}{r_{\max}} \quad (3\text{-}3)$$

Using equation(3-1), K_m and r_{max} could be obtained using regression. The calculated K_m was 1739 mg-N L^{-1}, and r_{max} was 769 mg-N·L^{-1}·d^{-1}. These results provide the optimum substrate concentration for subsequent research into efficient ammonia production systems. The proper substrate concentration should be at a value of twice the K_m, 3478 mg-N L^{-1}.

3.4.2. Effect of HRT on ammonia production

(1) Characteristics of ammonia production at various HRTs

Figure 3-3 demonstrates the time course of pH and TAN at various HRTs. The investigation of ammonia production for an HRT of 4 d was carried out in reactor H4, and HRTs of 2, 1 and 0.5 d were investigated consecutively during days 0-10, days 10-15 and days 15-18.25, respectively, in reactor H2. The organic nitrogen loading rates (ONLR) for HRTs of 0.5, 1, 2 and 4 d were 6956, 3478, 1739 and 867 mg-N·L^{-1}·d^{-1}, respectively. Previous work has shown that ammonia production at an HRT of 4 days runs well but shorter HRTs may risk failure of the whole process. The stepwise reduction of HRT could buffer the impact of shorter HRTs to the system and

allow the microorganisms to acclimate (Rajagopal et al., 2013). Thus, experiments with shorter HRTs of 2, 1 and 0.5 d were successively conducted in the same reactor (H2). All of the runs experienced at least 3 HRTs and reached steady state. Characteristics of ammonia production for various HRTs are summarised in Table 3-2.

According to Figure 3-3 (a), the pH in reactor H4 (at a HRT of 4 d) rapidly reached steady state at day 7 whereas the pH fluctuated in reactor H2. The pH at an HRT of 2 d reached steady state after 3 HRTs. The pH at an HRT of 1 d increased at first, suddenly decreased from 7.0 to 6.6, and finally recovered to approximately 6.8. The pH at an HRT of 0.5 d experienced a similar ascent-descent trend as well.

The TAN for HRTs of 4 d and 2 d reached the steady state after 3 HRTs; this pattern is coincident with their variances in pH. On the contrary, the TAN at an HRT of 1 d ascended initially and then considerably descended until it reached a steady state. Little variance of TAN was detected when the HRT was reduced to 0.5 d.

It was observed that the TAN increase occurred ahead of the pH decrease. TAN originates from the degradation of proteins. Proteins are hydrolysed with the release of ammonium and subsequently degrade to acidogenic products during anaerobic fermentation, which illustrates well why that the TAN increase occurred earlier than the pH decrease. The noticeable decrease of TAN at an HRT of 1 d should be ascribed to partial nitrification and denitrification in reactor H2 due to the occasional inclusion

of oxygen as the substrate was fed in. The pH increase that accompanied the TAN decrease also provides evidence of denitrification.

The steady state TAN value was maintained from day 12.25 until the end of the experiment even though the HRT was reduced to 0.5 d beginning on day 15. TAN grows with the increase of ONLR to some extent. This result indicates that microbes intended for ammonia production were maintained after the highest production rate was reached even though the ONLR was elevated by reducing the HRT. The degradation of VA led to an increase of pH at the end of the experiment. The constant value of TAN even during the shorter HRT (0.5 d) cycles accompanied with the increase of pH implies that the protein was incompletely degraded.

Table 3-2 listsed the TVA concentrations and VA spectral information for various HRTs at steady state. The TVAs for HRTs of 0.5, 1, 2 and 4 d were 12.7, 10.8, 10.9 and 8.8 g L^{-1}, respectively. The increase in TVAs was caused by increases in ONLR. The accumulated VA molecules could freely enter the microbial cells; once inside, the pH of the environment was lower and led to inhibition of the microorganisms. Hence, the excessive ONLR impacted the microorganisms and even led to the deactivation of microorganisms; in particular, the change affects methanogens, which are more sensitive to variations of ammonium and TVA. The impact on methanogens affects not only methanogenesis but also acidogenesis.

Acetic acid predominated in all four runs. The highest level of acetic acid was obtained at an HRT of 0.5 d. Acetic acid can be directly converted to CH_4 by methanogens unlike propionic, butyric or valeric acid. The accumulated acetic acid at an HRT of 0.5 d suggested a stunted methanogenic process. Methanogens are more sensitive to disturbances in anaerobic fermentation than acetogens (Montero et al., 2010). Although acidogenesis may not be affected in the short term by VA accumulation because acidogenic bacteria are more tolerant to toxins and higher ONLR, the levels will eventually deteriorate over long term operation.

This result is also supported by the lower VSS at an HRT of 0.5 d. The VSS values at HRTs of 0.5, 1, 2 and 4 d were 0.08, 0.13, 0.10 and 0.12%, respectively. The biomass decreased with the reduction of HRT.

(2) Kinetic analysis

The Monod equation has been successfully used to evaluate anaerobic fermentation kinetics (Ako et al., 2008). The Monod equation can be expressed as follows:

$$\mu = \frac{\mu_{\max} S}{K_S + S} \tag{3-4}$$

where K_s (g-N L^{-1}) is the half-saturation rate; S (g-N L^{-1}) is the residual substrate concentration at the steady state; and μ and μ_{max} are the specific bacterial growth rate (d^{-1}) and the maximum specific bacterial growth rate (d^{-1}), respectively.

Because the specific bacterial growth rate μ is equal to the dilution rate, the equation could be reformulated as

$$\frac{1}{D} = \frac{K_S}{\mu_{max}} \frac{1}{S} + \frac{1}{\mu_{max}} \tag{3-5}$$

where D is the dilution rate (d^{-1}).

By using this equation, the kinetic parameters can be calculated by regression (as shown in Figure 3-4).

The kinetic parameters K_s and μ_{max} were obtained from the Lineweaver-Burk plot (Figure 3-4). From the regression, μ_{max} was calculated as 1.7 d^{-1} with K_s equal to 501.1 mg-N L^{-1}.

(3) Simulation

The residual substrate balance is expressed as shown in equation(3-6):

$$\frac{dS}{dt} = D(S_0 - S) - \mu \frac{X}{\eta} \tag{3-6}$$

where X is the cell concentration (g L^{-1}-biomass) and η (g L^{-1}-biomass/mg L^{-1}-N) is defined as the microbes' growth yield. The biomass is represented by VSS. Thus, η could be expressed as follows:

$$\eta = \frac{X}{S_0 - S} \tag{3-7}$$

η was calculated by regression to be 0.074 g L^{-1}-biomass/mg L^{-1}-N.

S, X and r_b (the biomass growth rate in g·L^{-1}-biomass·d^{-1}) are expressed as follows:

$$S = \frac{K_S D}{\mu_{\max} - D} \tag{3-8}$$

$$X = \eta(S_0 - \frac{K_S D}{\mu_{\max} - D}) \tag{3-9}$$

$$r_b = DX = \eta D(S_0 - \frac{K_S D}{\mu_{\max} - D}) \tag{3-10}$$

S, *X* and r_b are functions of *D* for certain substrate concentrations. The relationship of *S*, *X* and r_b with *D* was demonstrated in .

According to , the critical dilution rate can be calculated as

$$D_{crit} = \mu_{\max} \frac{S_0}{K_S + S_0} \tag{3-11}$$

where D_{crit} is the critical dilution rate (d^{-1}), *i.e.*, wash-out occurs at D_{crit}.

Accordingly, the dilution rate D_m (d^{-1}) at the maximum productivity is required to be

$$D_m = \mu_{\max}(1 - \sqrt{\frac{K_S}{S_0 + K_S}}) \tag{3-12}$$

D_{crit} and D_m are calculated as 1.5 and 1.1 d^{-1}, respectively. In other words, microorganism wash-out and maximum productivity were predicted to be obtained at HRT of 0.6 and 0.9 d, respectively.

The model predicted the relationship between *S*, *X* and r_b and provides a reference for ammonia production during long-term operation. Compared to D_{crit}, a dilution level of D_m could facilitate the growth of microbes and enhance ammonia

production simultaneously. Therefore, a D_m corresponding to an HRT of 0.9 d should be used in further research.

3.5. Conclusion

In this section, an alkali-free system was developed to treat the liquid waste from food processing by anaerobic fermentation and simultaneously recover the ammonia/ammonium at an extremely low C/N ratio. The fixed bed reactor was used with the ammonia trap to develop the alkali-free system. Because ammonia recovery is considerably affected by the ammonium concentration of the broth, the optimum operation conditions for ammonium production were investigated in this part. The proper substrate concentration (shown in the form of nitrogen) for the ammonium production were obtained by the continuous operation and the kinetic analysis. According to the Michaelis-Menten model, the maximum ammonium production rate was achieved as 769 mg-N·L^{-1}·d^{-1}, corresponding to the substrate concentration as 3478 mg-N L^{-1}.

HRT is another crucial parameter for the ammonium production during the anaerobic process. Effects of HRT on the ammonium and ammonia production were studied by continuous operations and then simulated by Monod model. According to the kinetic analysis, the optimal substrate concentration was 1002 mg-N L^{-1} at an HRT

of 0.9 d. The microorganism wash-out was predicted to occur at an HRT of 0.6 d. An increase in ONLR led to VA accumulation at much shorter HRTs.

Table 3-1 Characteristics of ammonia production for various substrate concentrations at steady state

Reactor	TAN	VSS	pH	TVA	VA spectra (%)					
No.	(mg L^{-1})	(%)	-	(g L^{-1})	Acetic acid	Propionic acid	i-Butyric acid	n-Butyric acid	i-Valeric acid	n-Valeric acid
FSN1	1722	0.63	6.8	7.2	39.6	11.9	6.5	25.2	16.3	0.6
FSN2	1862	0.61	6.8	11.5	41.0	16.2	7.0	21.6	13.7	0.5
FSN3	2123	0.80	6.9	12.5	39.7	19.4	7.3	20.6	12.8	0.1
FSN4	1976	0.88	6.7	15.4	42.7	15.2	6.5	22.7	12.6	0.3

Table 3-2 Characteristics of ammonia production for various HRTs at steady state

No.	TAN	VSS	pH	TVA	VA spectra (%)					
	(mg L^{-1})	(%)	-	(g L^{-1})	Acetic acid	Propionic acid	i-Butyric acid	n-Butyric acid	i-Valeric acid	n-Valeric acid
HRT0.5	1518	0.08	6.8	12.7	50.5	16.6	6.0	15.0	10.3	1.6
HRT1	1475	0.13	6.7	10.8	41.2	17.0	6.3	21.4	12.8	1.3
HRT2	1799	0.10	6.8	10.9	31.1	15.4	6.4	31.6	14.5	1.1
HRT4	1913	0.12	6.9	8.8	39.9	17.9	5.7	20.8	14.6	1.1

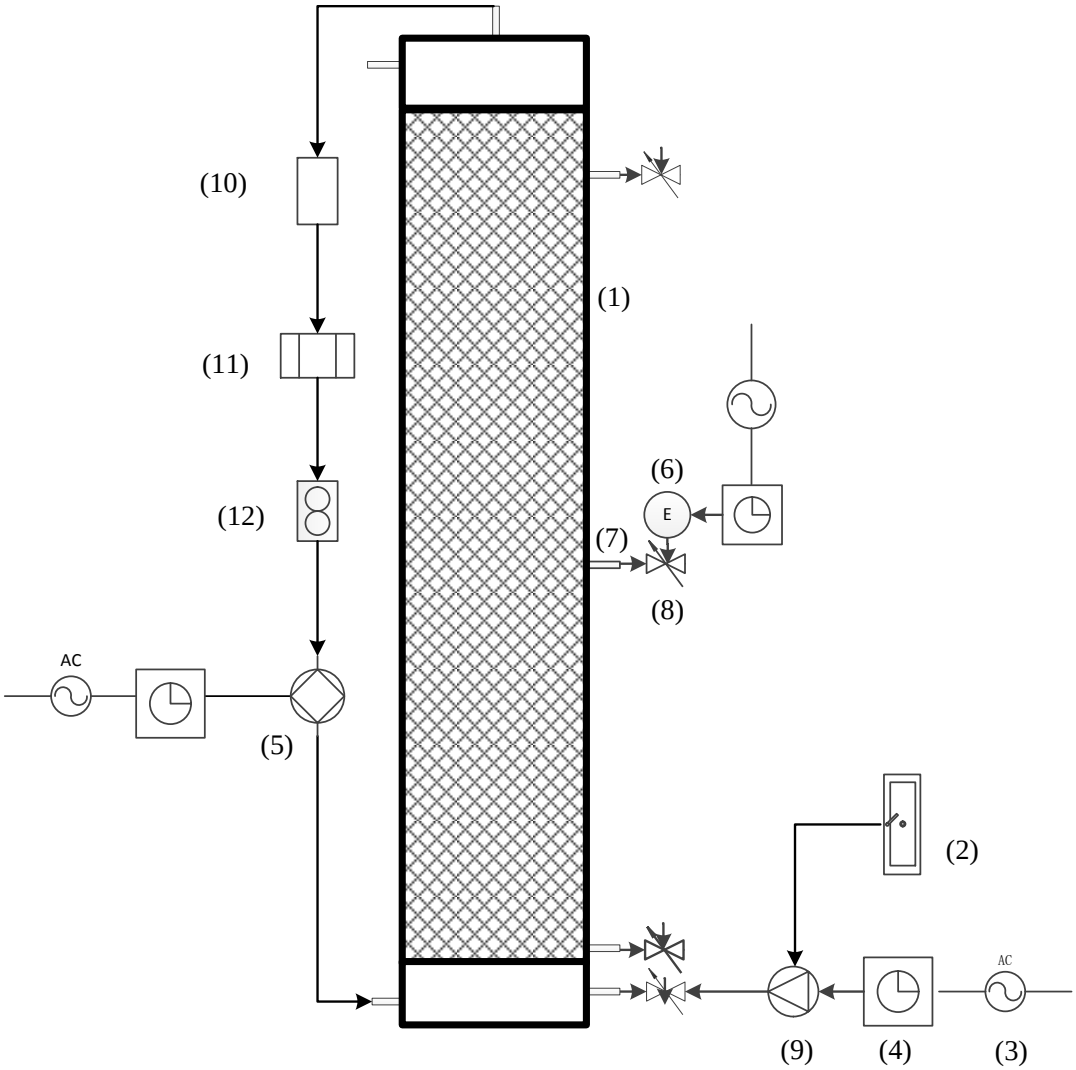


Figure 3-1 Scheme of the fixed bed reactor for ammonia production

(1) Fixed bed reactor; (2) Wastewater reservoir; (3) Power; (4) Timer; (5) Air pump;

(6) Magnetic valve; (7) Sample port; (8) Valve; (9) Peristaltic pump;

(10) Ammonia capture; (11) Gas collection; and (12) Flow meter.

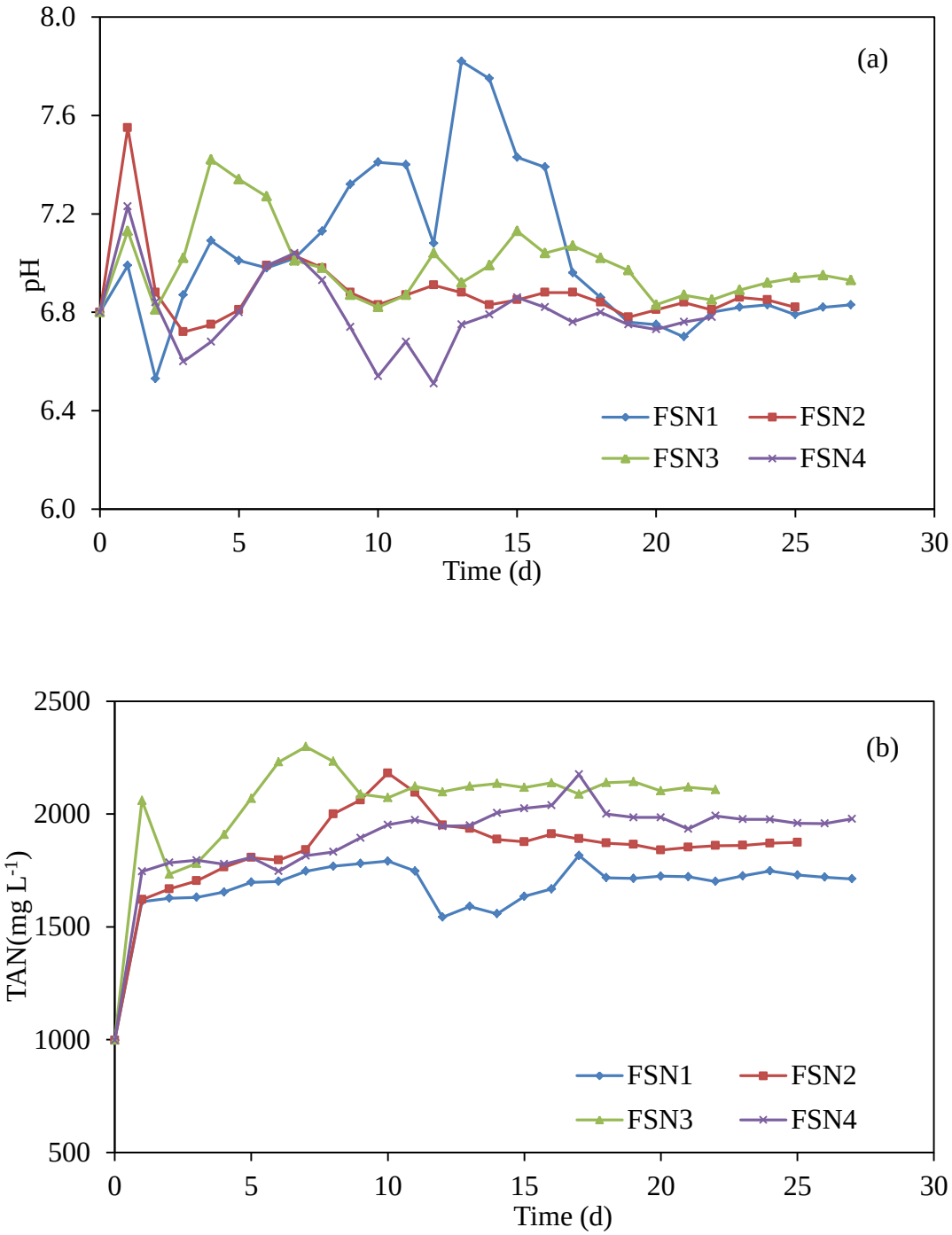
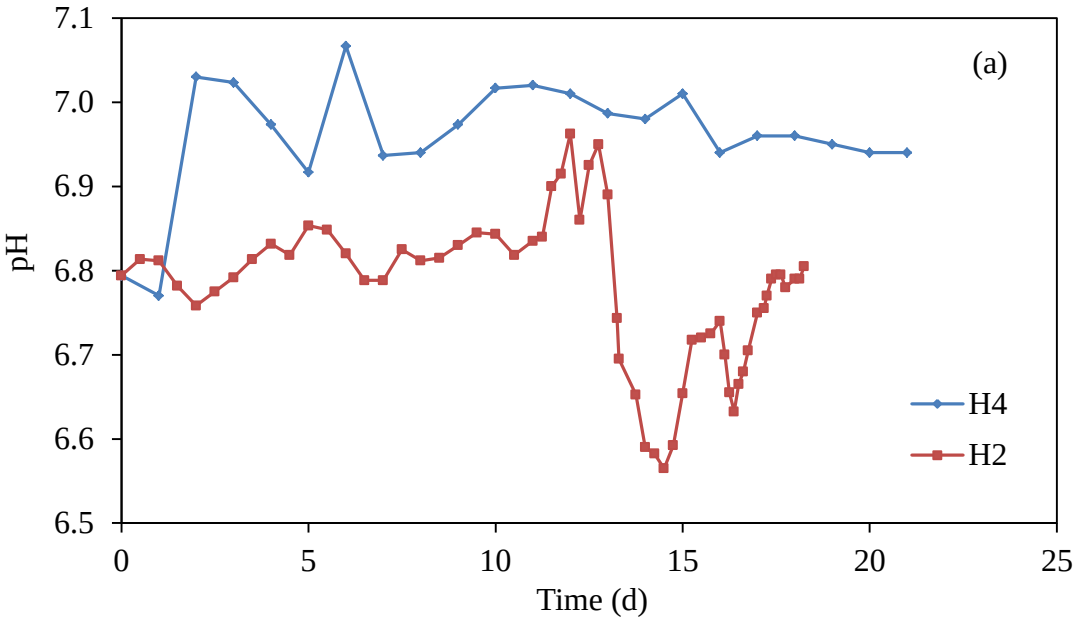


Figure 3-2 Time course of pH and TAN for various substrate concentrations



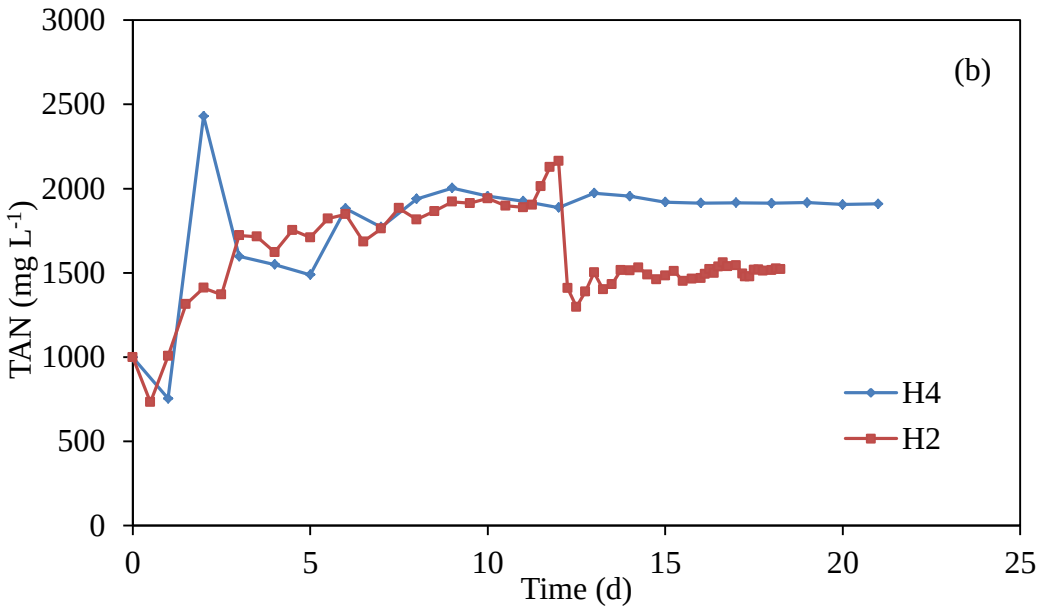


Figure 3-3 Time course of pH and TAN for various HRTs

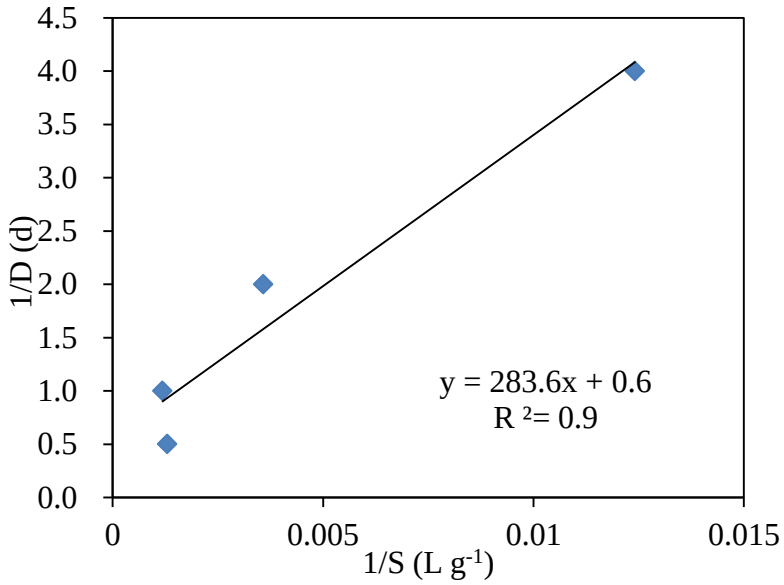


Figure 3-4 Lineweaver-Burk plot of ammonia production for various HRTs

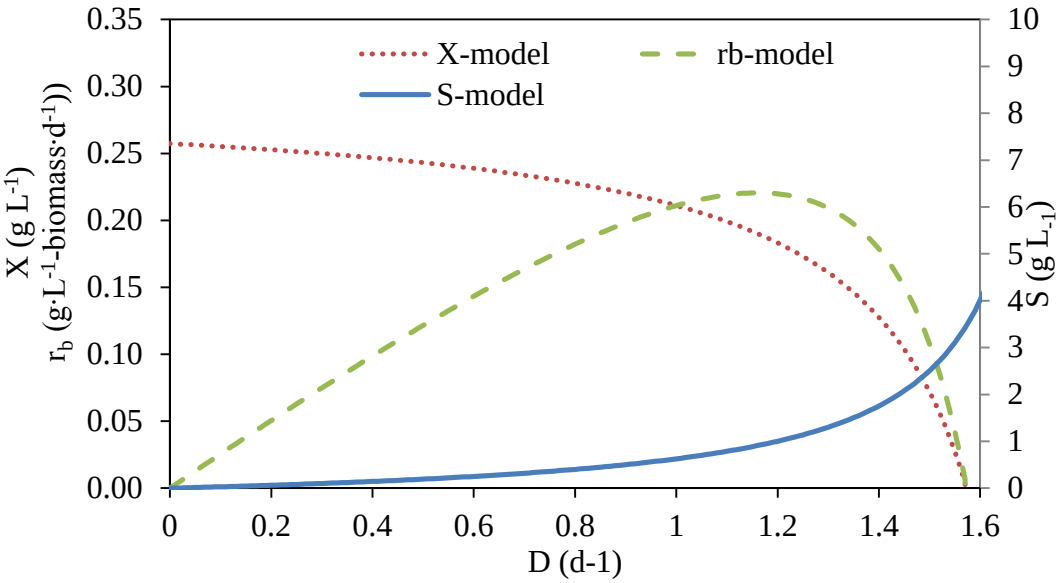


Figure 3-5 Kinetic simulations of substrate degradation and biomass growth at steady state

Chapter 4

Enhancement of organic conversion for liquid waste from food processing by the partial heating

4.1. Introduction

Liquid waste containing high strength nitrogenous concentration from the food processing has received increasingly attentions because its pollution to the environment. The excessive introduction of nitrogen compounds into the water body is one of the critical causes of eutrophication. Current treatment of the nitrogen-rich waste/wastewater including compost, CANON, SHARON and ANAMMOX (Vazquez-Padin et al., 2009; Kabore et al., 2010; Tang et al., 2010; Claros et al., 2012) are all based on the nitrification and the denitrification of the nitrogen compounds. The nitrogen compounds were converted to ammonium by an anaerobic process, followed by an aerobic process to produce nitrite and nitrate. The ammonium reacted with the produced nitrite/nitrate to remove nitrogen in the form of N_2. However, the greenhouse gas N_2O is prone to emission during the nitrification and the denitrification process especially at a low C/N ratio (Okabe et al., 2011; Sun

et al., 2013), which would intensify the global warming. In addition, bioenergy contained in the N-rich biomass has been wasted.

In fact, ammonium largely produced from wastewater treatment above mentioned is a kind of potential biomass energy because it is the precursory of ammonia. Ammonia is always known as a toxic because ammonia could permeate the membrane of microbes used in the waste biological treatment. That would hinder the microbes' metabolism and then result in a low treatment efficiency or even a failure. On the contrary, ammonia is widely used in both agriculture and industry. It is the second most used commercial chemical in the world (Jensen et al., 2007) as the raw material for fertilizer and cryogen., ammonia is also a carbon neural storage medium of hydrogen with the advantages of easy liquefaction and high energy density (Vitse et al., 2005). Besides, ammonia is a carbon neutral biofuel alternative with high energy density comparable with the petroleum energy (Klerke et al., 2008). Ammonia used currently is mainly produced from synthesis from fossil fuel when a great deal of CO_2 is emitted at the same time. Recently Ammonia recovery from an environmental-friendly way has been focused on. The recovery of ammonia from the waste treatment would reduce the excessive use of fossil fuel and alleviate the consequent global warming and energy challenge.

The recovery of ammonia from the anaerobic fermentation broth is mainly

determined by three factors, *i.e.* ammonium concentration, pH and temperature in the broth. Ammonium production in anaerobic fermentation is dependent on the C/N ratio of substrate and the inocula. Low C/N ratio of the substrate means the abundant nitrogen in raw materials and enhances the ammonium production to some extent (Zeshan et al., 2012); (Jiang et al., 2013). However, the extreme C/N ratio affects the sustainable anaerobic process and lead to the bio-reaction suspend. It is a challenge to successfully dispose the nitrogen rich stream with an extremely low C/N ratio. The inocula which have been gradually acclimated before inoculating are easy to adapt to the nitrogen-rich substrate and more tolerant to high ammonium production (Rajagopal et al., 2013). The adjustment of pH by alkali has been widely used to avoid the overly acidic condition and promote free ammonia stripping from the anaerobic system. Although pH adjustment can maintain the anaerobic fermentation of high strength nitrogenous substrate to some extent, overly alkali addition hinders the sustainable ammonium production and protein degradation Chen et al has witnessed that the protein degradation and conversion declined by approximately 24.6% at the pH of 8-10 compared to that at pH of 5-8 (Chen et al., 2012). High pH may destroy the origin microbial community structures and damage the cell activity (Hidaka et al., 2013). Besides, alkali addition increases the operation cost of the anaerobic fermentation in practical engineering. Temperature is another crucial

factor for ammonium production. Numerous researches proved that thermophilic process is preferred rather than the mesophilic one due to its achievement of higher loading rates of digestion and greater conversion of proteins (Yenigun and Demirel, 2013). Luo et al (2010) found that total ammonium nitrogen (TAN) production was increased by 30.1% and 77.7% at 60 and 70 ◦C, respectively, compared to that at 35 ◦C. Not only ammonium production is promoted by the thermophilic process, but also the ammonia volatilization is expected to be enhanced. Free ammonia concentration can be determined by in equation(4-1) (Bonmati and Flotats, 2003) and (4-2) (Turner, 1991; Kedziora et al., 2006).

$$[NH_3] = \frac{[NH_3 + NH_4^+]}{1 + \frac{[H^+]}{Ka}} = \frac{[NH_3 + NH_4^+]}{1 + 10^{pKa - pH}} \quad (4\text{-}1)$$

$$pKa = 4 \times 10^{-8} \times t^3 + 9 \times 10^{-5} \times t^2 - 0.0356 \times t + 10.072 \quad (4\text{-}2)$$

According to the equations above, higher temperature could facilitate free ammonia recovery. The recovery of ammonia from the anaerobic fermentation further helps the ammonium production and conversion due to the chemical equilibrium in the reaction system. However, the application of anaerobic fermentation at thermophilic temperature has been limited in practical engineering due to its high operation cost.

4.2. Objectives

In this work, a partial heating system was developed to produce ammonium by anaerobic fermentation of nitrogen-rich liquid waste and recover bio-ammonia at the same time. The objectives of this work are to (1) establish a partial heating system for the bio-ammonia production and recovery by anaerobic fermentation; (2) investigate the effect of partial heating modules on the ammonium production and ammonia recovery.

4.3. Materials and methods

4.3.1. Substrate and seeding sludge

The soluble protein powder (ORIHIRO, Tokyo, Japan) was used to prepare the synthetic wastewater as the substrate. The powder is derived from soy protein. The protein powder components are made by protein (81.2%), lipid 0 (1-5%), carbohydrate (2-12%). Besides, Na (0.43-1.2%), K (0.25%), Ca (0.80%), Mg (0.04-0.11%), Fe, (0.005-0.015%), Vitamin B1 (0.016‰), Vitamin B2 (0.018‰), Co (0.05 mg L^{-1}) and Ni (0.5 mg L^{-1}) are included in the synthetic wastewater as well. The total nitrogen (TN) of the substrate is 1009 mg-N L^{-1}. The C/N ratio for the synthetic wastewater is 3.9.

The seeding sludge was collected from a sewage treatment center in Ibaraki, Japan. The seeding sludge was acclimated by the synthetic wastewater mentioned above under HRT of 3 d at 35℃. The pH, total suspended solids (TSS) and volatile suspended solids (VSS) for the acclimated sludge were 6.8, 1% and 0.71%, respectively.

4.3.2. Experiment apparatus

The fixed bed reactor (shown in Figure 4-1) was employed in this work. The working volume of the fixed bed reactor is 2.6 L with Din=10 cm and H=50 cm. Pall rings, which are made of plastic and have diameters of 25 mm, were packed into the reactor. A ribbon heater (Heater Engineer, Tokyo, Japan) was used for the partial heating in this work. The ribbon heater wrapped fixed bed reactor. The height of the wrapped part of the fixed bed reactor is 6 cm. the ribbon heater and the thermal sensor (Sheathed thermocouple, Toyonetsukagaku Co.,Ltd, Japan) were connected to a temperature controller (TC-3000, AS ONE Corporation, Japan). The temperature sensor was inserted into the gap between the ribbon heater and the wall of the fixed bed reactor to respond to the temperature of the partial heating. The temperature of the partial heating was set at 60℃.

4.3.3. Experiment procedure

(1) Effects of single partial heating modules on the ammonia volatilization

Four fixed bed reactors were used to research the effect of varied single partial heating modules on ammonia production and recovery in this experiment. The one with partial heating was set as the control (marked as SR). The other three reactors whose ribbon heater was set at the top, middle and bottom of the reactor were marked as THSR, MHSR and BHSR, respectively.

The sample was taken from the middle sample port. Then the substrate was fed from the bottom of the reactor. The emitted biogas went through an ammonia trap (4M H_2SO_4) and was collected in a gas bag. The collected biogas was recycled into the reactor before sampling and after feeding, and mixed for 5 min at each phase at the rate of 2 L L^{-1} min^{-1}. Biogas mixing was performed for 60 min in total for one day.

The continuous operation was performed with HRT of 0.9 d at 37°C. Data was obtained after at least 3 HRTs to reach the steady state.

(2) Effects of dual partial heating module on the ammonia volatilization

The dual partial heating module was developed to enhance the production and volatilization of ammonia from anaerobic fermentation. The reactor performed with

dual partial heating module is marked as DSR in this work. According to the results of single partial heating modules, the combination of THSR and BHSR was adopted in this work.

The operation conditions were the same as that mentioned in 2.3.1.

4.3.4. Analytical methods

pH and TAN were monitored daily. TSS, VSS, total volatile acid concentration (TVA) and TN were measured during the steady state.

The pH was tested by a pH meter (TRX-90, TOKO, Japan), and TAN was tested by ammonia electrode (Ti-9000, TOKO, Japan). TSS and VSS test followed the standard method (APHA, 2005). TVA and VA spectra was tested by a gas chromatography (Shimadzu GC-14B, Japan) with an column (Unisole F-200) at the carrier gas (N_2) 40 mL min^{-1}. TN is detected by a TOC/TN analyzer (TOC-VCPH Shimadzu, Japan) with a nitrogen analyzer unit (TNM-1) with the carrier gas of pure air.

4.3.5. Parameter calculations

(1) Undegraded nitrogen (UN)

UN represents the nitrogen amount which has not been degraded. UN (mg L^{-1})

can be expressed by the following equation:

$$UN = TN - TNO_x - TAN \quad (4\text{-}3)$$

where TN (mg L^{-1}) is the total nitrogen in the broth; TNO_x (mg L^{-1}) is the nitrite and nitrate nitrogen in the broth; TAN is the NH_3/NH_4^+-N in the broth.

TNO_x in this work was so little that can be ignored. Thus, UN in this work can be calculated as follow:

$$UN = TN - TAN \quad (4\text{-}4)$$

(2) Ammonia volatilization ratio (AVR)

Ammonia volatilization ratio can be calculated by the equation as follows:

$$AVR = (TN_i - TN_t)/TN_i \times 100\% \quad (4\text{-}5)$$

where TN_i is the total nitrogen (mg L^{-1}) of the synthetic wastewater at the initial period and TN_t is the total nitrogen (mg L^{-1}) of the broth at the steady state.

(3) Ammonia recovery potential (ARP)

Only free ammonia in the aqueous can be recovered from the broth by chemical and physical methods. Thus, the ammonia recovery potential (ARP) can be calculated by equation(4-6):

$$ARP = [NH_3\text{-}N]/TAN \times 100\% \quad (4\text{-}6)$$

where NH_3-N (mg L^{-1}) and TAN (mg L^{-1}) are the free ammonia nitrogen

concentration and total ammonia concentration in the broth, respectively.

(4) Unionized VA concentration (UVA)

UVA content can be determined by equation(4-7):

$$UVA = TVA\frac{10^{(pKa-pH)}}{1+10^{(pKa-pH)}} \quad (4\text{-}7)$$

where TVA (g L^{-1}) is the total VA concentration of the broth, *pKa* is the acid dissociation constant in water; the *pKa* of acetic acid at 37 ℃ is 4.762.

4.4. Results and discussion

4.4.1. Effects of single partial heating modules

(1) Effects of single partial heating modules on NH_3/NH_4^+ bioproduction and nitrogen conversion

Time course of TAN was shown in Figure 4-2. TAN rapidly increased during the first day and gradually reached the steady state after 2.5 HRTs. The average TAN at the steady state was shown in Table 4-1. The average TAN for SR, THSR, MHSR and BHSR were 743.7, 777.6, 834.1 and 751.1 mg L^{-1}, respectively. The lowest TAN was obtained in SR as expected. The TN in the supernatant of broth for SR, THSR, MHSR and BHSR were 969.1, 804, 949.0 and 836.0 mg L^{-1}, respectively. TN in the supernatant of broth included the degradable dissolved protein (or amino

group), TAN and nitrogenous compounds in other forms. The reduction of TN in the liquid part of the broth was mainly caused by the volatilization of free ammonia and the consumption of microbes. TN used by microbes was ignored in this work because the concentrations of microbes were very low with VSS < 0.2% in all the runs. Thus the volatilization of free NH_3 became the main reason for the variances of TN According to the equation(4-1) and (4-2), free NH_3-N in the broth for SR, THSR, MHSR and BHSR should be 8.9, 7.2, 4.9 and 7.1 mg L^{-1}. In other words, the theoretical ARP in certain pH and temperature for this work ranged from 0.6% to 1.2%. However, the volatilized NH_3-N obtained in all experiments reached 39.9, 205.0, 60.0 and 173.0 mg L^{-1} in SR, THSR, MHSR and BHSR, respectively, meaning the AVRs in the four runs were promoted to the range of 3.3-16.6%. The high AVRs obtained in experiments were resulted from the continuous release of free ammonia, leading to the continuous chemical equilibrium transfer and protein degradation.

The enhancement of proein degradatioin can be proved by the results of UN (shown in Table 4-1). The lowest UN was attained in BHSR, followed by THSR and MHSR. UN in the control (SR) was highest in the four runs, taking 22.3% of the TN at the initial period. These results demonstrated anaerobic process with partial heating modules improved the ammonia transfer in the broth. In addition, the partial

heating module of THSR and BHSR sharply enhanced ammonia volatilization.

(2) Effects of single partial heating module on the acidogenesis

The acidogenesis of the nitrogen-rich substrate was evaluated by pH, TVA and VA spectra (present in Figure 4-1). Fermentation progress can be indirectly understood by the observation of acidogenesis because a sound acidogenic progress suggested a sustainable CH_4 production process. The pH varied in the range of 6.7-7.0. Lower pH in runs with partial heating modules may be caused by the free ammonia release when H^+ was accumulated in the broth.

TVA in the four runs varied in the range of 1.4 -2.8 g L^{-1}. The highest TVA was obtained in SR, followed by THSR and MHSR. The lowest TVA was achieved in BHSR. TVA of THSR, MHSR and BHSR were lower by 28.6%, 46.4% and 50.0% than that of SR, respectively. It seems that TVA has not reached the critical concentration of inhibition to the anaerobic process. The results of UVA were able to support the thoughts. According to equation(4-7), UVA amount in the four runs ranged from 0.6%-1.3%. The UVA is the toxic intermediate for the anaerobic process and considered as the main reason for the VA inhibition(Jiang et al., 2005; Chen et al., 2007). On the contrary, the ionized VA is ready for the conversion to gas by microbes. The low account of UVA in the broth suggested a good acidogenic process.

For the VA spectra, acetic acid dominated in each run. Acetic acid contents in runs with partial heating were higher than that of the control (SR). Propionic acid is believed to be the retardative VA in anaerobic fermentation, whereas it changed in the range of 1.3-9.9% in this experiment. Propionic acid concentration trended oppositely to acetic acid, which was consistent with previous findings that the propionic acid degradation rate increased in the case of low acetic acid content (Mawson, 1986; Mawson et al., 1991). The long chain VA (including the butyric acid and valeric acid) ratio in THSR was slightly lower than that of the control (SR), while the ones in MHSR and BHSR were lower by 29.6% and 21.4% compare to that of the control. The reduction of long chain VA in system with single partial heating modules suggestedthe enhanced conversion to acetic acid from the organic substrate. The high ratios of acetic acid in MHSR and BHSR is able to evident the phenomena.

(3) Effecst of single partial heating modules on the organic degradation and organisms

The total carbon (TC) removal ratio was present in Table 4-1. The carbon in SR, THSR, MHSR and BHSR reduced to 2402, 1511, 1706 and 1719 mg L^{-1} from the initial 3936 mg L^{-1}, respectively. The carbon removal ratio of THSR, MHSR and BHSR grew by 57.9%, 45.4% and 44.4% than that of SR, respectively, indicating

that partial heating modules obviously enhanced the carbon degradation. Similar researches also demonstrated that the organic degradation was enhanced at the thermophilic condition compared to the mesophilic condition (Kim et al., 2006; Lee et al., 2009). Kim et al (2006) found that there is a function relationship between temperature and the soluble chemical oxygen demand. Kim et al (2012) witnessed that the enzymes' activities of protein, lipid and amylum starch have been improved at the thermophilic environment.

In addition, the organic removal with single partial heating modules can be compared to other researches even though it is restricted by the low C/N ratio. The total organic carbon utilization of 83.9% at C/N ratio of 6.5 was reported (Wang et al., 2013). Zhong et al. (2013) has found that the organic removal ratio reached 42.2% in case of C/N ratio of 6. These results emphasized that C/N ratio of the substrate is a crucial controlling factor in the anaerobic fermentation.

The microbes' community structure may be also affected by the various partial heating modules. The effect of THSR seems not directly working on the microbes. The module of THSR created a concentration gradient at the height of the fixed bed fermentor, where the TAN concentration is lower at the top due to the enhanced ammonia volatilization compared to that of the bottom. The TAN would motion to the top from the bottom along the TAN concentration gradient. This process reduced

the TAN of the bottom, where the microbes concentrated. The reduction of production concentration enhanced the chemical equilibrium of the forward reaction. On the contrary, the BHSR made the suffering of the mesophilic microbes. Although the high temperature favored the hydrolysis and acidogenesis of the organics at the initial period of the fermentation, the methanogens might show negative activity at the thermophilic conditions.

4.4.2. Effect of dual heating module

(1) Effects of DSR on the NH_3/NH_4^+ production and removals

According to the results of single heating modules, THSR and BHSR presented better performance. The query of a dual heating module combining THSR and BHSR came out. Therefore, based on the investigation of effects of single heating modules on ammonium production and recovery, the dual heating module was developed. The performance of the dual partial heating module at the steady state was shown in Table 4-2.

TAN for DSR was 736.8 mg L^{-1}. Although TAN for DSR seems no higher than that of the SR, the AVR for DSR prevailed. The AVR for DSR was 18.4%. Compared to its theoretical ARP of mere 0.5% at the certain pH and TAN concentration, the ammonia volatilization has been considerably promoted by the

dual partial heating. Besides, the ARP for DSR was 5.6-folds of that of the SR, which also evidenced the enhancement of ammonia volatilization by dual partial heating considering the lower pH and TAN concentration in DSR.

UN in DSR was 92.7 mg L^{-1}, which was lower by 59.2% compared to that of the SR. UN in DSR was even higher than that those of THSR and BHSR, although their AVRs were similar. The phenomena suggested that the dual partial heating provided high ammonia volatilization efficiency in spite of the low pH in the liquid. Higher UN in DSR can be illustrated by the change of the heating module. Dual partial heating module may affect the temperature through the fixed bed reactor in comparison with the single partial heating modules. Organic degradation and acidogenesis can be improved at thermophilic conditions (Kim et al., 2006; Kim et al., 2012), which led to the decrease of pH and the subsequent retard of ammonia volatilization. The existence of ammonium and ammonia in the broth would be adverse to the conversion of protein in the anaerobic fermentation. Therefore, the UN in DSR did not prevail over the THSR or BHSR.

(2) Effects of DSR on the acidogenesis and organic degradation of nitrogen-rich wastewater

The average pH for the the DSR was 6.6, much lower than the control of 7.0. As shown in equation(4-1), NH_3 emission greatly depends on pH. Even small

variance of pH would result in great difference of NH_3 volatilization. The reason for pH decrease in DSR should be ascribed to the increase of TVA. TVA for the DSR was 3.6 g L^{-1}, which was 28.6% higher than that of the control. TVA exits in the anaerobic fermentation mainly in two forms, i.e. UVA and ionized volatile acid (IVA). pH was affected by IVA because of the VA ionization. On the contrary, microbes were mainly impacted by the UVA. UVA could pass through the cell membrane and cause the imbalance of intracellular and extracellular. The UVA concentration imbalance would lead to the microbes' activity decline and even to the fractures. The UVA in DSR took only 5% according to equation(4-7) , which was much lower than the threshold documented in previous studies. This result demonstrated that the disorder in the low C/N wastewater treatment for this work was not caused by the excessive UVA. In fact, higher temperature caused by dual partial heating favored the organics conversion and their acidogenesis rather than the methanogenesis (Kim et al., 2006; Lee et al., 2009). The methanogenesis was more stable at mesophilic condition (Bayr et al., 2012). The methanogenesis seems not to match the rapid acid production led by the higher temperature. In addition, the temperature control for the dual partial heating module should be improved in order to avoid the temperature increases through the fixed bed reactor in the future.

VA spectra for DSR was also present in Table 4-2. Although acetic acid

dominated in DSR, its content was lower than that of the control. Acetic acid is the precursor of methane for the acetate-consumed methanogens. Lower content of acetic acid in DSR suggested the conversion of VA to methane. Long chain volatile acids are produced from the degradation of complicated compounds. The long chain volatile acid concentration in DSR was higher by 73.8% and 152.7% than that of the SR and THSR, respectively. It indicated an effective acidogenesis of organics, but at the same time meant an imbalance between the rapid acidogenesis and acid consumption. Interestingly, the n-valeric concentration was much lower than that of i-valeric acid. Wang et al has found that the n-valeric acid was prone to degradation rather than i-valeric acid in anaerobic fermentation (Wang et al., 1999). The lower n-valeric acid content in DSR agreed with these findings as well.

TC removal ratio in DSR was 65.2%. Taken into the short HRT in this work, the TC removal efficiency is 2850.7 mg L^{-1} d^{-1}. The TC removal ratio was higher by 67.4% than that of the SR, while slightly higher than than of THSR. The results also evidenced the thoughts that the contrast of acid production rate and consumption rate decelerated the degradation of organics.

4.5. Conclusions

In this work, an anaerobic fermentation system with a partial heating system for

the bio-ammonia production and recovery has been developed. The effect of various heating modules on bio-ammonia production and organic degradation were investigated. For the single partial heating module, THSR and BHSR considerably prevailed over the control (without the partial heating). The ammonia volatilization ratio in THSR and BHSR were elevated by 5.0 and 4.0-folds compared to the control. The TC removal ratio in THSR in and BHSR were increased by 57.9% and 44.3%, respectively. Dual partial heating system showed similar performance as the single partial heating with respects to ammonia production and volatilization and the organic degradation. Results in this work demonstrated that the partial heating system considerably enhanced the organics removal and simultaneously recovered the potential valuable by-products from the wastewater at extremely low C/N ratio.

Table 4-1 Characteristics of the continuous operations at steady state

	SR	THSR	MHSR	BHSR
pH	7.0±0.1	6.9±0.1	6.7±0.0	6.9±0.1
TAN(mg L^{-1})	743.7±17.6	777.6±14.8	834.1±29.0	751.1±26.3
TN(mg L^{-1})	969.1±40.9	804.0±24.3	949.0±5.1	836.0±4.5
UN (mg L^{-1})	225.4	26.4	114.9	84.9
AVR (%)	3.3	19.8	5.3	16.6
TC removal ratio (%)	39.0	61.6	56.7	56.3
TVA (g L^{-1})	2.8	2.0	1.5	1.4
UVA (%)	0.6	0.8	1.3	0.8

Table 4-2 Characteristics of ammonia production in case of the dual partial heating module under the steady state

pH	TAN	TC removal ratio	TN	AVR	TVA	VA spectra (%)					
-	(mg L^{-1})	(%)	(mg L^{-1})	(%)	(g L^{-1})	Acetic	Propionic	i-Butyric	n-Butyric	i-Valeric	n-Valeric
6.6 ±0.1	736.8 ±31.8	65.2 ±0.5	829.5 ±17.7	18.4 ±0.9	3.6 ±0.3	52.1 ±1.6	9.7 ±0.9	6.9 ±0.6	11.7 ±0.7	19.6 ±2.4	N.D.

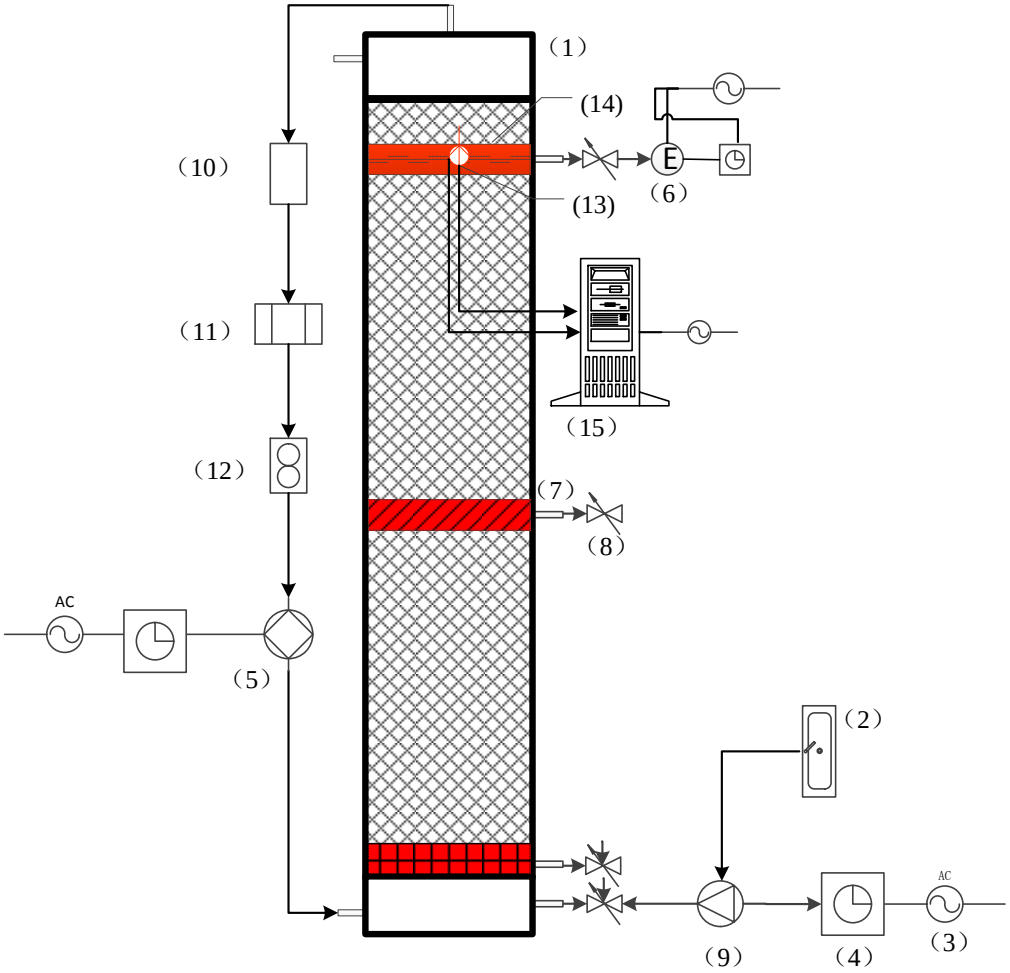


Figure 4-1 Schematic of ammonia production and recovery system

under various partial heating modules

Ribbon heater for THSR;

Ribbon heater for MHSR;

Ribbon heater for BHSR;

DSR means the double heating module, which is the combination of THSR and BHSR according to the results

(1)Fixed bed (2) Wastewater (3) Power (4) Time (5) Air pump

(6) Magnetic valve (7) Sample port (8) Valve (9) Peristaltic pump (10) Gas bag

(11) Flow meter (12) NH_3 trap (13) Temperature sensor

(14) Ribbon heater (15) Temperature controller

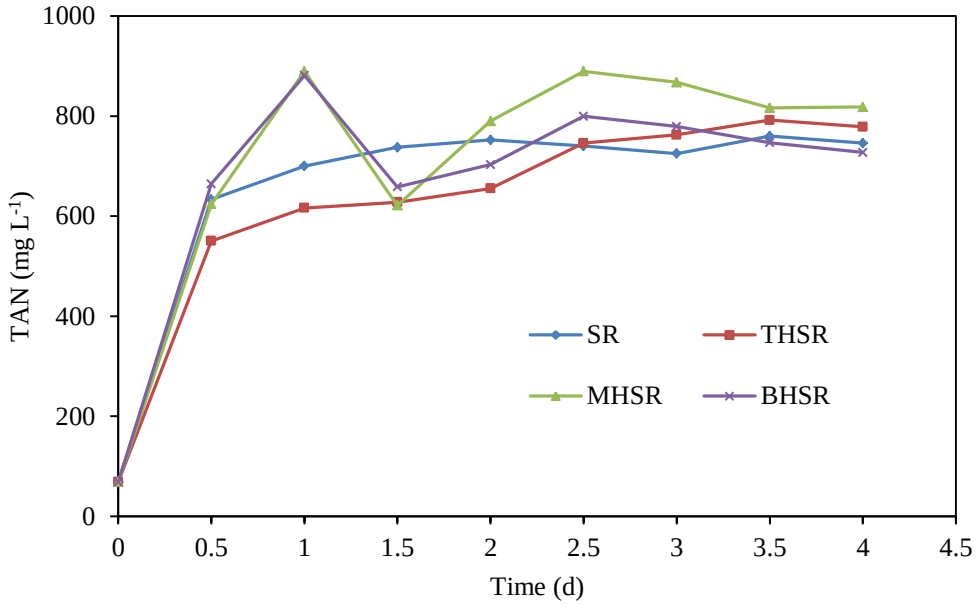


Figure 4-2 Time course of TAN for the control and runs with partial heating

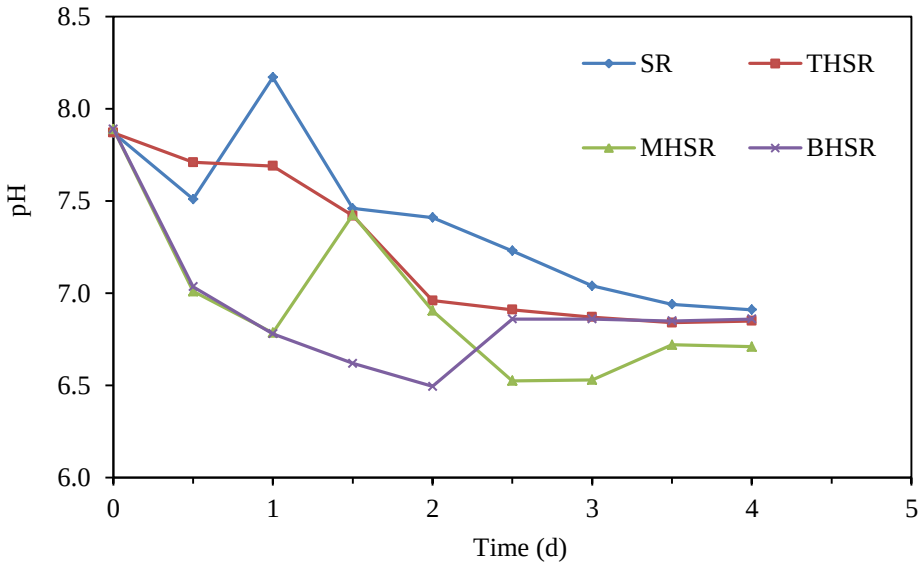


Figure 4-3 Time course of pH for the control and runs with partial heating

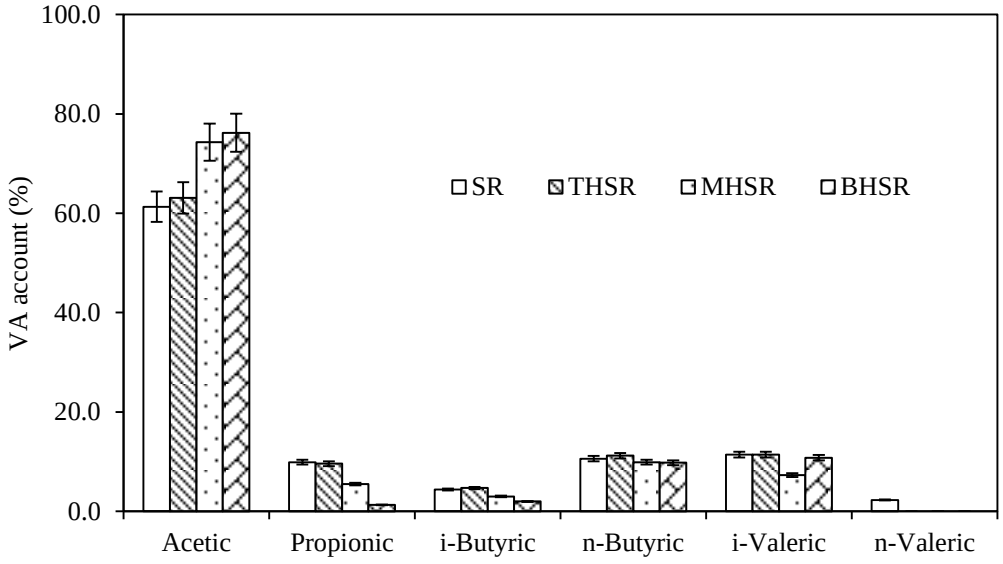


Figure 4-4 VA content for varied partial heating modules and the control

Chapter 5

Summary

5.1. Summary

Waste from food processing is produced in large quantities worldwide and contain high level of organics. The waste treatment and extraction of value-added products from the food processing waste can be achieved by anaerobic fermentation, however, the recalcitrance of the solid waste, and the large quantity and low C/N of the liquid waste have limited the energy and resource recovery from waste originated from food processing. Therefore, it is necessary to develop effective bioreactors for the anaerobic fermentation of solid and liquid waste from the food processing, respectively to enhance the bioenergy recovery and value-added products extraction.

In this work, bioreactors targeting anaerobic fermentation of solid and liquid waste from food processing were developed, respectively, considering the substrate characteristics of the anaerobic process. The overall conclusions were presented as follows.

A simultaneous pretreatment and acidogenesis process for solid waste from food processing has been developed by an RDF system with methanogenic leachate recirculation. The enhancement of simultaneous pretreatment and acidogenesis was achieved by methanogenic leachate recirculation and WRS addition. In the continuous operations, methanogenic leachate recirculation significantly increased hydrolysis rates and volatile solids (VS) degradation. The VS degradation ratio and the hydrolysis rate constant at a higher leachate recirculation ratio (2:1 weight ratio of methanogenic leachate to substrate) were increased by 2.1- and 1.4-fold, respectively, compared to those of the lower ratio (1:1 leachate recirculation ratio). A 10% (weight ratio of WRS to substrate solid content) WRS addition assisted the biochemical reactions in the process at the higher leachate recirculation ratio was employed. WRS could to adsorb part of the VA and alleviate the inhibition caused by VA, which improved the performance of the whole anaerobic fermentation. The hydrolysis rate constant and VS degradation were elevated by 54.7% and 63.9%, respectively, with the WRS addition. Besides, the WRS addition enhanced the VA formation and its conversion to biogas.

For the liquid waste from food processing with extremely low C/N ratio, an alkali-free system was developed to recover biogas and the bioammonium from. A kinetic model was performed to predict ammonium concentration and the optimal

conditions for ammonium production in anaerobic fermentation. Characteristics of bioammonium production using anaerobic fermentation were obtained by continuous operations. According to the kinetic simulation, the optimum substrate concentration was 1002 mg-N L^{-1} at an HRT of 0.9 d. This result meant that the bioammonia production by anaerobic fermentation could be achieved in a very short cycle and made the rapid production and recovery possible. The microorganism wash-out was predicted to occur at an HRT of 0.6 d, which was consistent with the experiment results. The increased organic nitrogen loading rate led to VA accumulation at extremely short HRT.

Furthermore, a partial heating system has been developed to treat the liquid waste from food processing. Effects of various partial heating modules on bio-ammonia production and organic degradation were investigated. The system with single partial heating modules prevailed over the control (without partial heating module). The highest ammonia volatilization rate and total carbon removal ratio obtained in single partial heating module were elevated by 5.0-folds and 57.9% compared to the control, respectively. Anaerobic fermentation system with the dual partial heating module showed similar performance as the single partial heating modules with respects to ammonia production and volatilization and the organic degradation. Results demonstrated anaerobic fermentation system with partial

heating modules considerably enhanced organics removal and simultaneously recovered potential valuable by-products from the wastewater at extremely low C/N ratio.

5.2. Outlook

Characteristics of the anaerobic fermentation of the solid and liquid waste from food processing have been studied respectively in this work. In fact, the effluent from the SPA contains abundant acetic acid, ammonium and little solid, which should be deeply treated by the fixed bed fermentor to be recover the bioenergy. Therefore, the combination of the rotational drum fermentor and the fixed bed fermentor is proposed with practical necessity and the technical feasibility. The conceive for the combination system is shown in Figure 5-1.

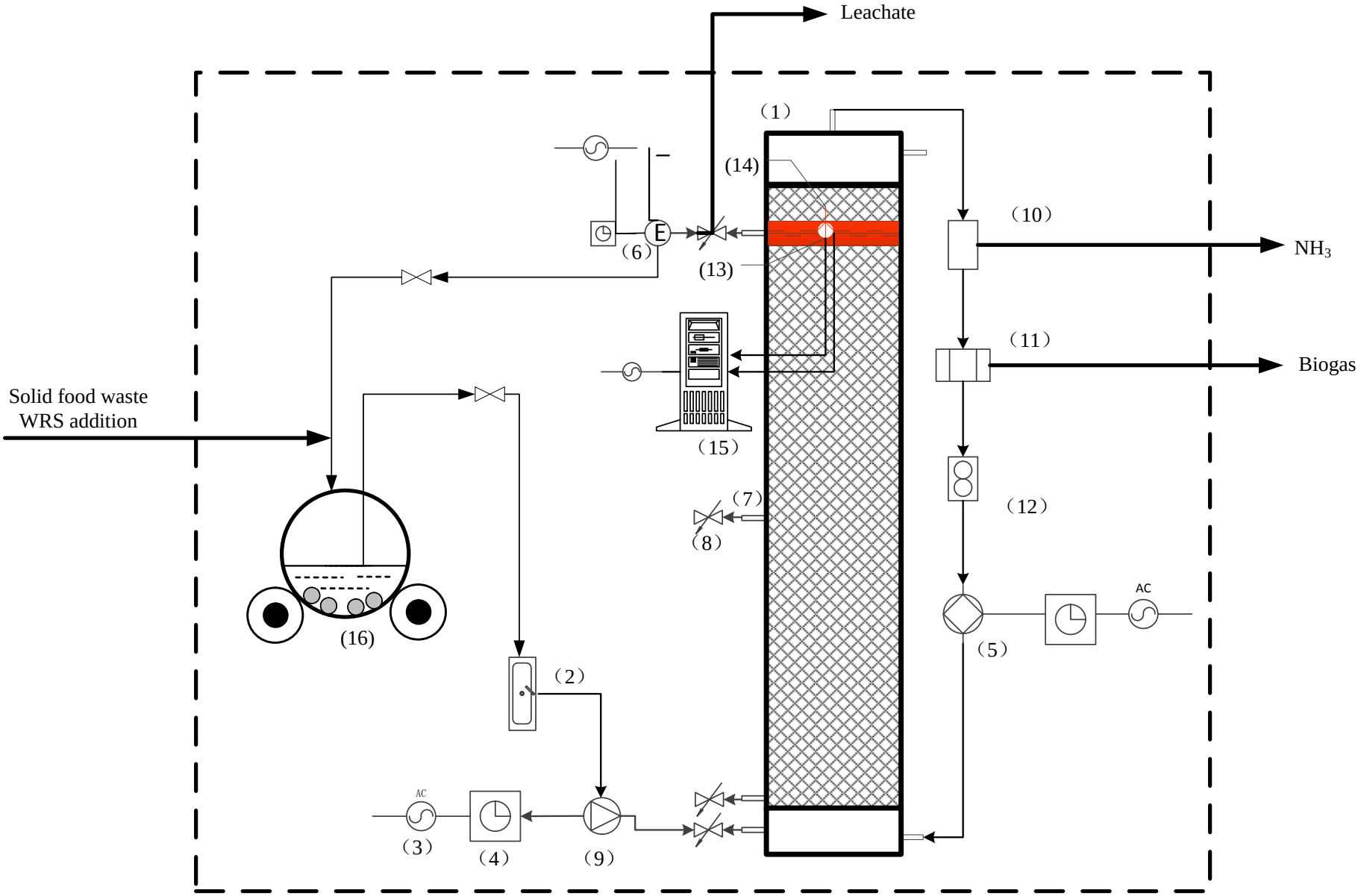


Fig. 5-1 The conceive for the combination system for the anaerobic fermentation of the solid and liquid food processing waste

Legends:

(1)Fixed bed fermentor

(2) Wastewater

(3) Power

(4) Time

(5) Air pump

(6) Magnetic valve

(7) Sample port

(8) Valve

(9) Peristaltic pump

(10) Gas bag

(11) Flow meter

(12) NH_3 trap

(13) Temperature sensor

(14) Ribbon heater

(15) Temperature controller

(16) Rotational drum fermentor

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