Master's Thesis

Effect of thermal pretreatment on chemical composition and biogas production from kitchen waste

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ABSTRACT

Biogas has been well known with its wide range of benefits in terms of greenhouse reduction, energy security, creating jobs and generating revenue. However, biological conversion of several biogas feedstocks to biogas under anaerobic digestion has proven inefficient due to low conversion efficiency. Pretreatment of certain type of biomass has been known to improve the biodegradability and methane yield of the pretreated biomass. In this study, the effect of thermal pretreatments viz., pressure-cooking with steam (PC1), pressure-cooking with water (PC2), steam pretreatment in steamer and hydrothermal pretreatments on chemical composition and methane potential of kitchen waste was investigated. Grab samples of kitchen waste were collected from Yliotö restaurant located on the Ylistönrinne campus of the University of Jyväskylä. Methane production from untreated and pretreated kitchen waste was determined in batch experiments at 35 °C and 55 °C.

Chemical analyses showed that untreated kitchen waste, which was mainly composed of vegetables trimmings (56%), fruit skins and spoilt fruit (43%) and bread (1%), had total solids (TS) content of 12.6 % with volatile solids (VS)/TS ratio of 0.94. PC1, PC2 and steam pretreatment resulted in an increase in TS and VS by 1.6-20.2 % and 0.8-21.1 %, respectively. On the other hand, TS and VS content of hydrothermally pretreated kitchen waste remained more or less unchanged. All pretreatments resulted in an increase in soluble chemical oxygen demand (SCOD), ammonium nitrogen (NH_4-N) and total kjeldahl nitrogen (TKN) concentration. However, complete loss of NH_4-N was noticed in PC2. Loss of volatile fatty acid (VFA) (41.5-85.7%) was also noticed from all pretreated samples due to high pretreatment temperature used in the study. From untreated kitchen waste, methane yields of 426 ml/$gVS_{original}$ at 35 °C and 452 ml/$gVS_{original}$ at 55 °C were obtained. Similarly, methane yields obtained from pretreated kitchen varied from 410-439 ml/$gVS_{original}$ at 35 °C and 406-462 ml/$gVS_{original}$ at 55 °C.

The results indicate that the studied thermal pretreatment methods had affected the chemical characteristics of kitchen waste to a large extent but no or little effect on methane yields. An increase in methane yields of 1.9-3.1 % was noticed in pretreatments viz., PC1, PC2 and steam pretreatment. The low or no increase in methane yield was probably due to loss of VFA during thermal pretreatment and the possibility of inhibitory products formation.

TIIVISTELMÄ

Energiaomavaraisuus, kasvihuonekaasupäästöjen väheneminen, sekä työllisyyden lisääntyminen usein luetaan biokaasuntuotannon hyödyiksi. Hyödyistä huolimatta, orgaanisen aineksen anaerobinen käsittely ei ole ongelmatonta. Prosessitehokkuutta haittaa usein syötemateriaalien koostumus etenkin ligniinin osalta, joka sellaisenaan on prosessissa heikosti hajoavaa. Tässä työssä tutkittiin, termisiä esikäsittelyt vaikutus (painekeittäminen höyryllä ja vedellä, höyryllä ja hydroterminen esikäsittely) keittiöjätteen kemiallisesta koostumukseen ja biokaasupotentiaaliin. Näihin menetelmiin lukeutuvat termiset esikäsittelymenetelmät, kuten käsittely painekattilassa ja hydroterminen esikäsittely.

Tutkittavat jätteet on kerätty Jyväskylän yliopiston kampusravintola Ylistöstä ja ne sisältävät pääasiassa vihannesten perkeitä (56 %), hedelmien kuoria ja pilaantuneita hedelmiä (43 %), sekä leipää (1 %). Keittiöjätteen kuiva-ainepitoisuus (TS) oli 12,6 % ja orgaanisen kuiva-aineen pitoisuus (VS) 94 % (% TS:stä). Esikäsittely painekattilassa lisäsi TS ja VS pitoisuutta 1,6-20,2 % ja höyrystimessä 0,8-21,1 %. Hydrotermisellä esikäsittelyllä ei ollut merkittävää vaikutusta TS- ja VS-pitoisuuksiin. Kaikki esikäsittelymenetelmät lisäsivät keittiöjätteen kemiallista hapenkulutusta (SCOD), ammonium typen pitoisuutta (NH_4-N), sekä kokonaistypen pitoisuutta (TKN). Käsittelyn painekattilassa huomattiin laskevan NH_4-N pitoisuutta merkittävästi. Korkean lämpötilan huomattiin myös pienentävän haihtuvien rasvahappojen (VFA) pitoisuutta merkittävästi esikäsitellyissä näytteissä. Käsittelemättömän keittiöjätteen metaanipotentiaali oli 426±14 ml/gVS 35 °C:ssa ja 452 ml/gVS 55 °C:ssa. Esikäsitellyissä ruokajätenäytteissä metaanipotentiaali vaihteli 410-439 ml/gVS välillä 35 °C:ssa, sekä 406-462 ml/gVS välillä 55 °C:ssa.

Tulokset osoittavat että eri esikäsittelymenetelmät vaikuttavat merkittävästi jätteen kemiallisiin ominaisuuksiin, mutteivät niinkään keittiöjätteen metaanipotentiaaleihin. Tämän uskotaan johtuvan prosessissa syntyvien haitta-aineiden vaikutuksesta tai merkittävästi pienentyneestä haihtuvien orgaanisten ainesten pitoisuudesta ja menetys haihtuvien rasvahappojen (VFA) pitoisuutta.

TABLE OF CONTENTS

ABBREVIATIONS

BMP	Biochemical Methane Potential Test
C/N ratio	Carbon/Nitrogen ratio
CH_4	Methane
EU	European Union
FID	Flame Ionization Detector
FM	Fresh Matter
Free NH_3	Free ammonia
HRT	Hydraulic Retention Time
MSW	Municipal Solid Waste
NH_4-N	Ammonium nitrogen
NH_4^+	Ammonium ion
OLR	Organic Loading Rate
PC1	Pressure-cooking with steam
PC2	Pressure-cooking with water
SCOD	Soluble Chemical Oxygen Demand
TKN	Total Kjeldahl Nitrogen
TS	Total Solids
TVFA	Total Volatile Fatty Acids
VFAs	Volatile Fatty Acids
VS	Volatile Solids
w/w	Wet weight

1 INTRODUCTION

1.1 Background

World energy demand is increasing from time to time as a result of burgeoning population and expansion of high-energy intensive industries (Aduba et al. 2013). Till today, the majority of this energy is coming from petroleum (36.3 %), coal (20.2 %) and natural gas (24.5 %) being the main fossil energy sources (International Energy Agency 2010). The European commission study shows that over the 20th century, world fossil fuel and material consumption has increased by a factor of 12 and 34, respectively (European commission 2011). Therefore, as human population continues to expand, it is obvious that the demand for fossil fuels also keeps on increasing and it will not take too long for fossil fuels to diminish (Riddell et al. 2004). Due to these above reasons and the negative impacts of fossil fuels on environment and human health, world has been looking for renewable energy sources (Aduba et al. 2013).

Renewable energy provides a wide range of benefits in terms of greenhouse reduction, energy security, creating jobs for local communities and also generates income (Kasper 2013). In Europe, its development and application has been increased due to the EU's commitment to reduce the greenhouse emissions and its impact on climate change (Europa 2007). Among several renewable energies, biogas is one of the most promising sources of energy (Katuwal & Bohara 2009). It can be produced from a wide range of feedstock mainly from most biomass and organic municipal waste materials (Aduba et al. 2013).

The rate of municipal waste generation is increasing rapidly than emissions of greenhouse gases and other environmental pollutants as a consequence of population growth and improvement of living quality (Hoornweg et al. 2013). Daniel & Perinaz (2012) in the report published by World Bank warned that global municipal solid waste (MSW) generation rate is expected to increase from 1.3 to 2.2 billion tonnes per year by 2025. According to European Environment Agency, 280 million tonnes of MSWs were generated in the European Union in 2010. Similarly, in Finland, the amount of MSW generated in 2010 was 2.5 million tonnes (European Energy Agency 2013). Of which, 1.1 million tonnes was landfilled, with 70% of MSW being the biodegradable waste (European Energy Agency 2013). Ending MSWs at landfill sites or combusting them in in-

cinerator are not the best methods of waste management due to methane and other organic compound emissions to the environment (Scheuer 2005). According to EU directive 1999/31/EC, the amount of biodegradable municipal waste being landfill must be reduced.

Among several waste treatment techniques such as composting, incineration and landfilling, anaerobic digestion is the most cost–effective waste treatment method, due to high-energy recovery and low environmental impact (Cave 2013). The energy recovered during anaerobic digestion can be used to generate electricity and heat, or can be upgraded to natural gas quality and used as a transportation fuels (Antognoni et al. 2013). The digestate left after biogas production can be used source of fertilizer and/or for composting (Chen 2010).

1.2 Biochemistry and microbiology of anaerobic digestion process

Anaerobic digestion is a biological process where organic materials (e.g. carbohydrates, protein and fats) are degraded by microorganims under anaerobic condition to biogas (50-70 % of methane and 30-40 % of carbon dioxide), water vapour and other impurities such as hydrogen sulphide, nitrogen and carbon monoxide (Wilkie 2005, Ray et al. 2013).

The four main steps of anaerobic digestion are hydrolysis, acidogenesis, acetogenesis, and methanogenesis and are shown in Figure 1. In the hydrolysis step, the particulate organic matter i.e. proteins, carbohydrates and fats are transformed to amino acids, monosaccharides and long chain fatty acids respectively by the extra cellular enzymes produced by the facultative and obligatory anaerobic microorganisms. In the acidogenic phase, the soluble organic matter and the products of hydrolysis are converted into organic acids (C1-C5 molecules), alcohols, hydrogen and carbon dioxide by the obligatory anaerobic acidogenic bacteria. During the acetogenesis, the acetogenic bacteria convert the products of acidogenesis into acetic acid, hydrogen and carbon dioxide. Acetogenic bacteria are hydrogen producers and survive only at low hydrogen partial pressure ($< 10^{-5}$ bar) (Schink 1997). For this reason, acetogenic bacteria live in symbiosis with methanogenic microorganisms. In the methanogenesis step, methanogenic bacteria (Domain: Archaea) are responsible for methane production from the products of acetogenesis under strict anaerobic conditions (Velmurugan & Ramanujam 2011). About 70

% of methane is formed from acetate (Acetoclastic methanogenesis) and the remaining 30 % is directly from hydrogen and carbon dioxide (hydrogenotrophic methanogenesis) (Kavuma 2013).
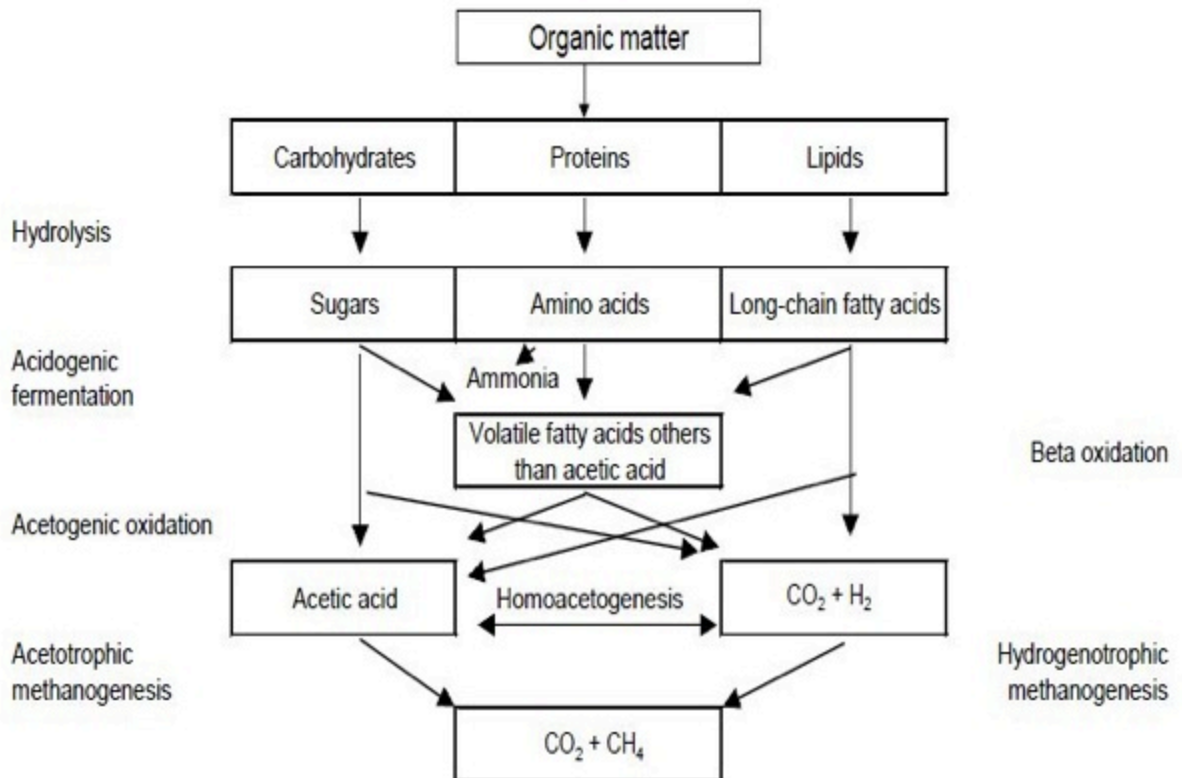
Figure 1. Biochemistry of anaerobic digestion of complex organic compounds (Adapted from Rapport et al. 2008).

All the above steps should work together for a better digestion process, because the first step provides substrate required by the next steps. For example, if hydrolysis is inhibited, the substrate needed by the second, third and fourth steps will be affected and as a result there is a reduction in methane production (Liqian 2011). In addition, the methanogens are the most sensitive microorganisms to process parameters, such as pH, temperature, and substrate concentration. These methanogen have very slow growth rates (doubling time of 5–25 days) and thus, methanogenesis is usually the rate-limiting step (Youngsukkasem et al. 2013).

1.3 Factor affecting anaerobic digestion of organic wastes

Biological conversion of organic materials to biogas under anaerobic condition is a complex process, which highly affected by different factors (Nelson 2011). Among several factors, the most important parameters are temperature, pH, toxic materials, total

solid, type of substrate, carbon/nitrogen ratio, organic loading rate, hydraulic retention time, ammonia, volatile fatty acids and etc. (Rodriguez-Marthnez et al. 2005, Lu et al. 2006, Nelson 2011, Harilal et al. 2012).

Anaerobic digestion produces methane within a wide range of temperature from psychrophilic (< 20 °C) to mesophilic (29 to 41 °C) and thermophilic (49 to 60 °C). However in practice, mesophilic and thermophilic processes are commonly used for continuous methane production, especially for industrial applications. The quality of biogas produced out of the recommended temperature ranges is low, because anaerobic bacteria (specially thermophilic bacteria) are sensitive to temperature change (Harilal et al. 2012).

The recommended pH interval for anaerobic digestion is between 7 and 8 with a small variation. If the pH goes over and below the suggested range, the activities of microorganisms will be highly affected. This means that highly acidic or highly alkaline conditions will have a great impact on methane production. However, at acid forming stage, the pH might be too acidic (below 6), but at the final stage of anaerobic digestion pH value will rise to 7, because methanogenesis bacteria is sensitive to acidic environment (Harilal et al. 2012).

Total solid content is another physical factor that can affect the process of anaerobic digestion. If the total solid content of bioreactor is too low, it means that its too dilute and the solid particles can precipitate at the bottom of the reactor. Similarly, if there is high amount of total solid present in the reactor, the flow of gas can be limited. Therefore, the slurry mixture is neither too diluted nor too thick for proper solubility of organic materials (Karki et al. 2005).

The presence of carbohydrate, fat, proteins and minerals in the digester are important for the microorganism development and methane production. Theoretical methane production potential of carbohydrates, proteins, and lipids are 415, 496 and 1014 ml/gVS, respectively (Angelidaki & Sanders 2004). However, overfeeding of these substrates might inhibit degradation process due to accumulation of ammonia and long chain fatty acids (Liqian 2011). The composition and characteristics of the feedstock, in generally, affect the digestion time of anaerobic process. It might take several weeks to complete

the digestion of highly lignin contain materials than simple sugars, volatile fatty acids, and alcohols (Aduba et al. 2013).

Hydraulic retention time (HRT) is another factor, which indicates the minimum time that the substrate stays within the reactor (Navona 2010). In many cases, HRT for mesophilic and thermophilic process ranges from 35 days to 50 days (Harilal et al. 2012). If the HRT is shorter, the slowest growing microorganisms and nutrient, which are necessary for the anaerobic process, will be wash out and affect the process. Longer HRT will give enough time for the substrate to interact with bacteria, but it requires large size digester (Nelson 2010).

Organic loading rate (OLR) is also an important factor especially for continuous anaerobic digestion process, because it indicates the amount of organic matter added to biogas reactor per time. High OLR is one of the main reasons for accumulation of volatile fatty acid that drop the pH of the system and affect the activity of methanogenic bacteria (Nayono 2010).

The presence of carbon and nitrogen in biogas substrate are essential for growth of anaerobic bacteria. However, high or low amount of carbon/nitrogen (C/N) ratio in the substrates affect anaerobic digestion process due to high nitrogen consumption or due to high accumulation of nitrogen in the form of ammonia and thus subsequently inhibit methanogens. However, co-digestion of substrates with high and low C/N ratio will benefit the process through optimal nutritional conditions. In general, the optimal C/N ratio suggested for anaerobic digestion is 20-30:1 (Resch et al. 2011, Leke et al. 2013).

The presence of toxic materials such as mineral ions, heavy metals and detergents easily affect anaerobic digestion process. The presence of digested slurry also considered as toxic material if it is not removed in time, because it contains mineral ions like sodium, potassium, copper, nickel, calcium, magnesium and sulpahte that affect the normal growth of bacteria (Harilal et al. 2012).

Ammonia is an essential nutrient for microorganism development and produced through biological degradation of nitrogenous matter. However, high ammonium ion (NH_4^+) and free ammonia (NH_3), which are the two principal forms of inorganic ammonia nitrogen, inhibit anaerobic digestion of substrate. Free ammonia is a membrane permeable so that hydrophobic ammonia molecule enters the cell and cause proton imbalance or potassi-

um deficiency (Chen et al. 2007). Among several factors, pH and temperature highly affect ammonia inhibition during anaerobic digestion process. For instance, high pH increases the concentration of free ammonia to ionized NH_4^+ and as a result increases the toxicity. Similarly, increasing process temperature results in a higher concentration of free ammonia even though it has a positive effect on the metabolic rate of the micro-organisms. The recommended ammonia concentration for anaerobic process is about or below 200 mg/l (Chen et al. 2007). Adding water can dilute ammonia concentration of organic materials, which might require big size digestion reactor (Nelson 2010).

Volatile fatty acids (VFAs) are short chain fatty acids with a carbon chain of C1-C6. It mainly includes acetic acid, propanoic acid, and butyric acid, which are easily volatilize into air depend on the temperature. Methanogenic bacteria require VFA as intermediate product for metabolic pathway of methane production. However, high concentration of VFA, which can be the result of overloading of excessive fat containing materials and high ammonia concentration, drops the pH of anaerobic digestion and lower methane production (Nelson 2010).

1.4 Anaerobic digestion of kitchen wastes

Now a days, the generation of kitchen waste is increasing due to population growth and urbanization. As a result, kitchen waste management is one of the biggest challenges. In many places, kitchen waste is disposed off to the environment or ended up at landfills with others municipal waste, which later causes many problems to the public and environment (Sunil & Narayan 2013). On the other hand, it is a promising feedstock for energy production (Salunkhe 2012).

Among several waste-to-energy conversion technologies, anaerobic digestion is the most suitable bioconversion technologies for kitchen waste (Zhang et al. 2007), because it contains highly biodegradable materials, high moisture content and lots of nutrients for microbes (Ben-yi & Jun-xin 2006). Jayalakshmi et al. (2007) reported that about 90% of biodegradable part of kitchen waste is suitable for anaerobic digestion. However, the biodegradability rate varies depending upon the composition and chemical characteristics of kitchen waste.

According to previous studies, kitchen/food waste has high potential for methane production both under mesophilic and thermophilic conditions. Gunaseelan (2004) studied

the methane potential of fruit and vegetable at 35 °C and found 180-732 ml/gVS and 190-400 ml/gVS of methane, respectively after 100 days of digestion. Similarly, Heo et al. (2004) also reported that methane potential of Korean traditional food waste, which contains boiled rice (10-15 %), vegetables (65-70 %), and meat and egg (15-20 %), was 489 ml/gVS at 35 °C after 40 days of digestion. Furthermore, Neves et al. (2008) study showed that methane potential of restaurant waste under mesophilic conditions varied from 400 to 490 ml/gVS.

There are also other studies that investigated the methane potential of food/kitchen waste under thermophilic condition. According to Zhang et al. (2007), the methane potential of restaurant food waste was 435 ml/gVS at 55 °C after 28 days of digestion. Similarly, Banks et al. (2008) evaluated the biogas potential of domestic food waste under mesophilic (36.5 °C) and thermophilic (56 °C) temperatures and reported 630 ml/gVS and 670 ml/gVS, respectively. In addition, Chen et al. (2010) reported the biogas potential of different biomasses under mesophilic and thermophilic conditions and they found that the final biogas yield obtained from commercial kitchen waste was 600±40 ml/gVS at 35 °C and 740±100 ml/gVS at 55 °C. Therefore, food/kitchen waste is one of the main feedstock for biogas production due to its high moisture and nutrient content for microbes.

1.5 Methods to enhance biogas production

High biogas yield is important for economic viability of the biogas plant. However, some biogas production from some feedstock results in low biogas yields (Bauer et al. 2007). The main reasons for low methane yields from some feedstock could be due to recalcitrance of the biomass (high resistant against anaerobic digestion), due to their compact and complex structure, or presence of inhibitors. The degradation of complex materials is a slow process and is usually limited by the hydrolysis phase of anaerobic digestion (Sims 2013).

Pretreatment techniques improve the degradation rate and efficiency of anaerobic digestion, and also improve the bioavailability of the feedstock (Kumar et al. 2009). To be effective, pretreatment should increase the surface area of the substrate to make the carbohydrates more accessible for enzymes, minimize the loss of carbohydrates during the pretreatment, increase the recovery of lignin degradation into useful products, limit the

formation of inhibitors compounds and reduces energy demand (Hendriks & Zeeman, 2009).

Different feedstocks require different pretreatment methods depending on their structure and composition. The choice of a suitable pretreatment method is however depends on the biomass properties, optimal pretreatment condition for biogas process, economical as well as environmental issue (Guo 2012). As shown in Figure 2, pretreatment can be divided into: mechanical, thermal, chemical, biological and combination of them (Wyman 2013).
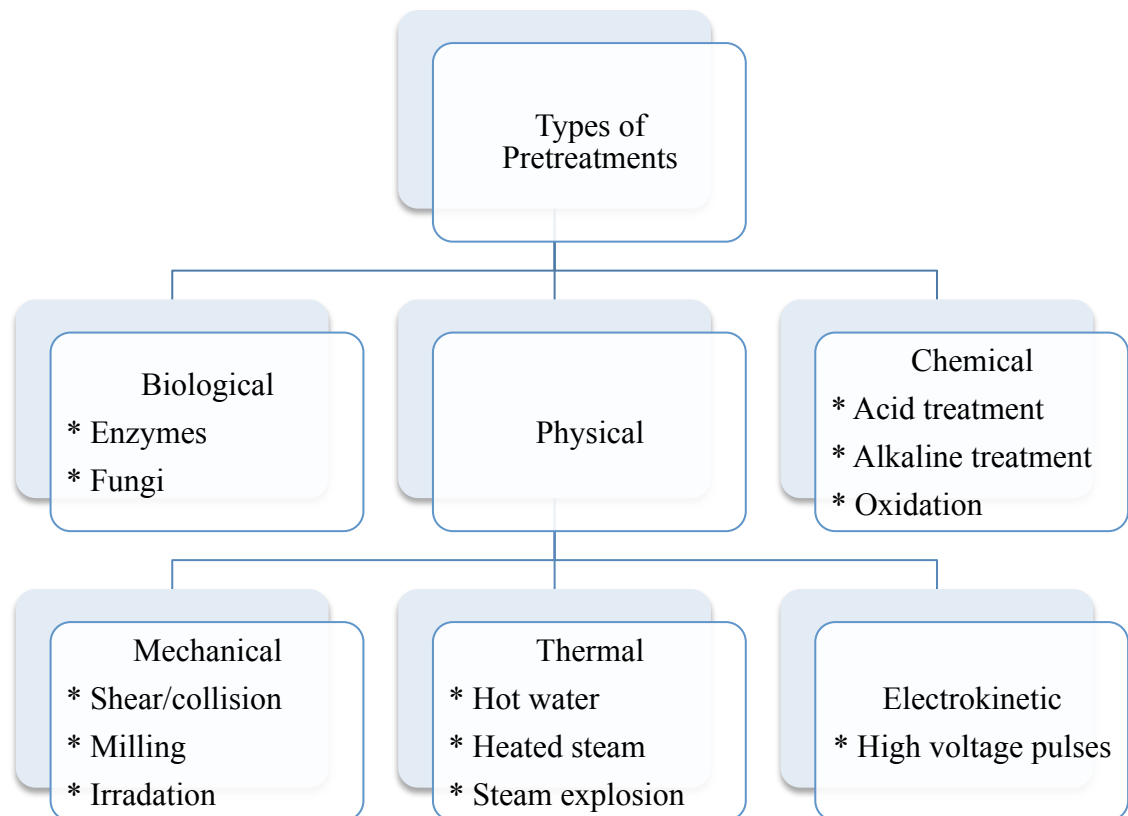
Figure 2. Pretreatment techniques of lignocellulosic materials (Modified from Taherzadeh & Karimi 2008).

Mechanical pretreatment is a physical process mainly used for of size reduction by chipping, grinding or milling. During size reduction process, the crystallinity of the materials and degree of polymerization highly reduced and as a result the available surface area for enzyme degradation increase. The main limitation of this technique is, its energy demand, but the formation of inhibitory compounds is low (Raju et al. 2012). Irradiation (gamma rays, electron beams, or microwaves) is also another mechanical pretreatment, which increases the accessible surface area and the pore size of the material, and also reduces the crystalline structure (Taherzadeh & Karimi 2008).

Thermal pretreatment is a process of heating lignocellulosic materials to a certain temperature and pressure in order to destroy the structure of the material. Among several thermal pretreatment techniques, steam, liquid hot water, autohydrolysis, and aquasolv pretreatments (Triantafyllidis et al. 2013) are widely used to improve the biodegradability of the substrates (Taherzadeh & Karimi 2008). The main limitation of this technology is the formation of inhibitors such as furfural and soluble phenolic compounds, which inhibit the production of methane (Hendriks & Zeeman 2009). As it mentioned by Liqian et al. (2011), at a temperature of 160 °C, thermal pretreatment degrade both hemicellulose and lignin. This degradation of lignin produces inhibitors such as phenolic compounds. The formation of inhibitors during liquid hot water pretreatment is relative low due to high water input (Sims 2013). In order to minimize the formation of these inhibitors, addition of external alkali might be needed to keep the pH around 4 to 7 (Hendriks & Zeeman 2009).

Chemical pretreatment mainly includes acid and alkaline pretreatment. Acid pretreatment can be classified into dilute and concentrated acid pretreatment under high and low temperatures, respectively. Some of the acids used under acid pretreatment are sulfuric acid, nitric acid, hydrochloric acid and phosphoric acid that effectively remove hemicelluloses and/or solubilize lignin. Alkaline pretreatment uses sodium hydroxide, potassium hydroxide, calcium hydroxide, aqueous ammonia and ammonium hydroxide to increase cellulose accessibility by solubilizing lignin and hemicellulose (Taherzadeh & Karimi 2008).

In biological pretreatment, microorganisms viz. brown, white and soft rot-fungi are used to enhance the biodegradability of organic matter by degrading lignin and hemicellulose. The efficiency of microorganism to degrade cellulose is very low, because cellulose is more resistance than hemicellulose and lignin (Taherzadeh & Karimi 2008). This method requires low-capital cost and low energy demand, but it takes long resident times due to low biological hydrolyzing rate (Hendriks & Zeeman 2009).

1.6 Thermal pretreatment of kitchen waste

Kitchen waste contains a variety of materials such as food waste, fruits and vegetables, egg shells, bone, tea bags, coffee grounds, tissue papers, meat packing materials and etc. with different physical and chemical characteristics. All these materials are not suit-

able for anaerobic digestion because some of them are not easily biodegradable and some are toxic. Therefore, pretreatment is necessary prior to anaerobic digestion to remove impurities and to improve the biodegradability of the materials (Ramzan et al. 2010). Among several pretreatment technologies mentioned above, this study focuses on different thermal pretreatment techniques such as steam and liquid hot water.

Many researchers have studied the effect of thermal pretreatment on biogas production from kitchen/food waste and their results were varied based on substrate composition and process conditions. Marin & Kennedy (2010) studied the effect of microwave irradiation on biodegradability of kitchen waste and reported that the solubilization of source separated kitchen waste was improved by 40 % when treated at 175 °C (heating rate of 7.9 °C/min). They also reported that the soluble chemical oxygen demand of the substrate was increased after thermal pretreatment. Ma et al. (2011) also conducted study on the effect of thermal pretreatment (at 120 °C for 30 minutes) on chemical composition and methane yield of kitchen waste. They reported that the pH was more or less unchanged but others parameters such as total solids, volatile solids, soluble chemical oxygen demand and methane were increased by 5.4 %, 4.5 %, 24 % and 10 %, respectively.

However, in some cases, thermal pretreatments did not improve the biogas production rate of a certain materials and even decreased the final biogas yield of anaerobic digestion (Liqian et al. 2011). Liu et al. (2012) investigated this by analyzing the physical and chemical properties of thermally pretreated (at 175 °C for 60 minutes) kitchen waste, vegetable/fruit residue, and waste activated sludge. Their results show that thermal pretreatment increased the methane potential of waste activated sludge by 34.8 % but decreased the methane potential of kitchen waste and vegetable/fruit by 7.9 % and 11.7 %, respectively due to melanoidin production. Furthermore, dewatering of the waste activated sludge was improved, soluble chemical oxygen demand, soluble sugar, and soluble protein were increased, but the viscosity was significantly decreased. According to Ariunbaatar 2013, thermal pretreatment (at 80 °C for 1.5 hour) increases methane yield of food waste by 28 %. However, as the pretreatment temperature increased over 80 °C, the methane yield was significantly reduced due to loss of volatile organic or formation of melanoids (Ariunbaatar 2013). Similarly, Dlabaja & Malaťák (2013) also evaluated the effect thermal pretreatment (at 70 °C for 70 minutes) on chemical

characteristics and biogas yield of mechanically crushed kitchen waste. There results showed that the pH and total solids/volatile solids ratio of thermal pretreated kitchen waste more or less unchanged. However, the total solids was increased by 4.9 % after thermal pretreatment and biogas yield was decreased by 1.7 % after 20 days of anaerobic digestion at 35 °C. According to the above authors, the main reason is that the majority of the material was already cooked and no need for further pretreatment to avoid the formation of unwanted products.

As it can be observed from previous studies, the effect of different thermal pretreatment techniques on methane yield vary depends on pretreatment conditions such as pretreatment temperature, holding time and pH.

1.7 Objective

The aim of this study was to evaluate the effect of thermal pretreatments on the chemical composition and methane potential of kitchen waste.

2 METHODS AND MATERIALS

2.1 Origin of materials

2.1.1 Kitchen waste

Grab samples of kitchen waste was collected for two days (13-14.05.2013) from Yliotö restaurant located on the Ylistönrinne campus of the University of Jyväskylä, Finland. As a preliminary step, the collected kitchen waste was manually segregated into different fractions of waste. Later, it was shredded twice in a meat mincer (Talsa W22) to ensure uniform particle size (< 2 mm) and to homogeneous the substrate (Figure 3). The homogenized kitchen waste was then stored at +4 ^{0}C until used and after a week the sample was stored at -20 ^{0}C until the end of the study.

Figure 3. Manually sorted kitchen waste before (left) and after shredding (right) (Photo: Gossa Wordofa).

2.1.2 Inocula

For batch experiments, two different inocula were used. For thermophilic experiments, thermophilically digested material from a full-scale biogas plant (Stormossen) treating putrescible organic fraction of municipal waste (Vaasa, Finland) was used as inoculum. For mesophilic experiments, digested material from a farm-scale biogas plant (Kalmari Farm, Laukaa) treating cow manure, industrial confectionery waste and some energy crops at 35 °C were used. Prior to use, inocula were incubated at the desired temperature to reactivate microorganisms and minimize background methane production.

2.2 Thermal pretreatment

In this study, pressure-cooking, steam and hydrothermal pretreatment were analyzed. The homogenized kitchen waste was pretreated by steam and water in a pressure cooker, steamer (juice extractor) and hydrothermal reactor.

2.2.1 Pressure-cooking

Pressure-cooking pretreatment was carried out in a domestic pressure cooker (Ultra Endura+, India) with a total volume of 2 liter. The pressure cooker is designed to operate at maximum temperature of 130 °C and at constant pressure of 0.8 m^3/kg. The constant pressure is maintained by venting the excess pressure through the vent located on the gasket fitted lid. In the present study, two different pressure-cooking methods were used: i) pressure cooking with steam (PC1) and ii) pressure cooking with water (PC2).

In case of PC1, 70 g of kitchen waste was placed on a perforated tray, which was placed in the pressure cooker (Figure 4). Then, approximately 210 ml of water was transferred to the pressure cooker to generate steam. The steam generated during the pressure-cooking was used to pretreat the substrate at a constant pressure of 0.8 m^3/kg for 5 minutes (> 100 °C). It should be noted that the kitchen waste was not in contact with the water.

Figure 4. PC1 before (left) and after (right) pretreatment (PC1: Pressure-cooking with steam) (Photo: Gossa Wordofa).

Similarly, in case of PC2, 210 ml of water and 70 g of kitchen waste mixed and transferred to the pressure cooker (Figure 5). The material was cooked at the same constant pressure of 0.8 m^3/kg for 5 minutes (> 100 °C).
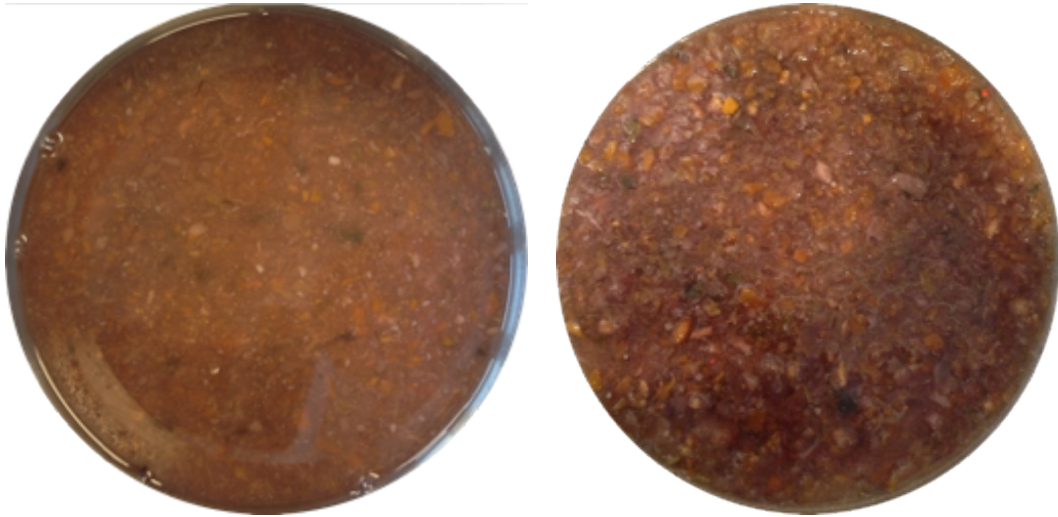

Figure 5. PC2 before (left) and after (right) pretreatment (PC2: pressure-cooking with water) (Photo: Gossa Wordofa).

2.2.2 Steam pretreatment

Steam pretreatment was carried out in a domestic Mehu-Liisa steamer/juicer extractor (Opa Oy, Finland) with a total capacity of 14 liters (Figure 6).
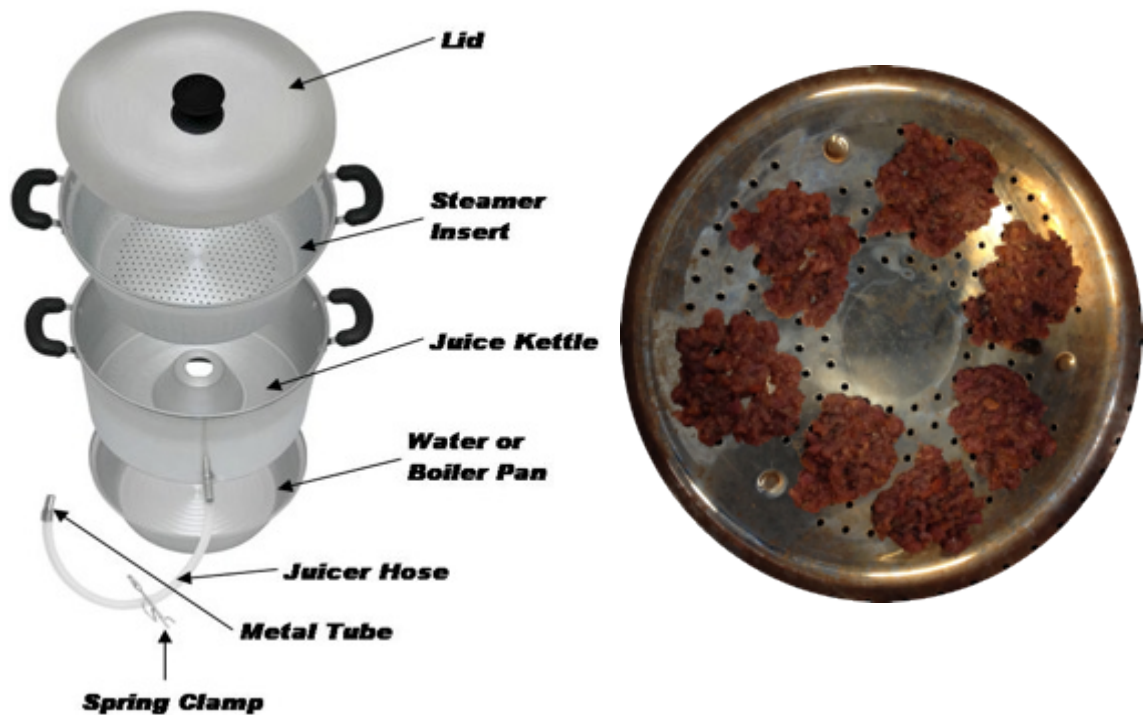

Figure 6. Steamer/juice extractor set up (left) and sampling method (right) (Photo: Gossa Wordofa).

It contains three different parts: perforated fruit basket (7.7 l capacity), juice kettle with hosepipe (capacity 2 l) and bottom water pot for steam generation (4.3 l capacity). At first, the steamer/juice extractor with sufficient amount of water (about 3.2 l) was brought to simmer. The sample to be cooked was then placed in a fruit basket. The hot steam circulates through the pot and cooks the sample for 30 minutes. After 30 minutes of cooking, the sample was cooled down to the room temperature and then the condensed steam was collected from the juice kettle through hosepipe and mixed with the pretreated sample on fruit basket. The whole system was made airtight as possible to avoid any loss of steam.

2.2.3 Hydrothermal pretreatment

Hydrothermal pretreatment was performed in a high temperature (250 °C) and pressure (250 bars) reactor (Berghof with Berghof DTR 841 heating system, Germany) (Figure 7). For each pretreatment cycle, 70 g of substrates (w/w) and 210 ml of water was used. Reactor contents were flushed with pure nitrogen (100 % N_2, Aga). The pretreatment conditions were 100 °C for 30 minutes. After pretreatment, substrate was left to cool down still next day.

Figure 7. Hydrothermal reactor (Photo: Gossa Wordofa).

The summary of the thermal pretreatment conditions is presented in Table 1. In all pretreatment methods, kitchen waste to water ratio was 1: 3, except that of steam pretreatment. After pretreatment, the pretreated and untreated samples were characterized immediately and/or stored at +4 °C until further use.

Table 1. Summery of pretreatment operation conditions.

Treatment methods	Temperature °C	Pretreatment time (min)	Kitchen waste to water ratio
PC1[*]	100-130	5	1:3
PC2	100-130	5	1:3
Steam[*]	100	30	-
Hydrothermal	100	30	1:3

Note: * sample was not in contact or mixed with water. PC1: Pressure-cooking with steam, PC2: Pressure-cooking with water.

2.3 Batch digestion tests

The methane potential of pretreated and untreated kitchen waste was studied in batch experiments using 120 ml glass bottles with working volume of 60 ml (Figure 8). To each assay, 20 ml of inoculum, 10 g substrate (w/w) and 30 ml of water was added (Table 2).

Table 2. Batch experimental set-up.

Samples	Sample (gFM)	Inoculum (gFM)	Water (ml)	Working Vol. (ml)
Untreated kitchen waste	10	20	30	60
Pretreated kitchen waste				
PC1	10	20	30	60
PC2	10	20	30	60
Steam	10	20	30	60
Hydrothermal	10	20	30	60
Inoculum/blank	-	20	40	60

Note: PC1: Pressure-cooking with steam, PC2: Pressure-cooking with water

The prepared assays were flushed with 30 % carbon dioxide (CO_2) and 70 % nitrogen for 3 to 5 minutes and sealed with butyl rubber stoppers and aluminum crimps. Two sets of 18 assays were prepared for each temperature and incubated in triplicate at 35 °C

and 55 °C. Assays with inoculum alone were used as control and the methane produced from the controls was subtracted from the sample assays.
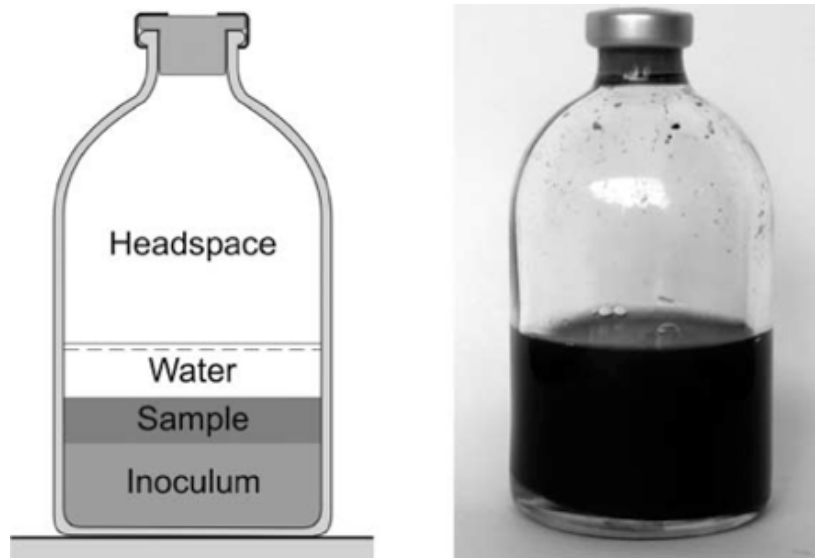

Figure 8. Schematic diagram of batch experiments for biogas production (Adapted from Angelidaki et al. 2009).

2.4 Analysis and calculations

The chemical compositions of kitchen waste such as pH, total solids (TS), volatile solids (VS), ammonium nitrogen (NH_4-N), total Kjeldahl Nitrogen (TKN), soluble chemical oxygen demand (SCOD) and volatile fatty acids (VFA) were measured from untreated and pretreated kitchen waste.

The pH of the sample was measured with pH meter (Methrohm 774). TS and VS were analyzed according to standard SFS 3008 (Finnish Standard Association 1990). NH_4-N and TKN were measured by using a Kjeltec 1002 distillation unit (Tecator AB) and according to protocol published by Perstop Analytical/ Tecator AB (1998). SCOD was analyzed from filtered sample according to Finnish standard SFS 5504 (Finnish Standard Association 1988). VFA were analyzed by a gas chromatograph equipped with FID (Perkin-Elmer Autosystem XL, using Perkin-Elmer Elite FFAP column 27.5 m x 0.32 mm with film thickness 0.25 μm) as described by Bayr et al. (2013). The values of each individual VFA components were converted to mgSCOD/l by multiplying with constant conversion factors. These conversion factors are: 1.066 for acetic acid, 1.514 for propionic acid, 1.818 for butyric and iso-butyric acid, 2.039 for valeric, iso-valeric and caprioc acid. Finally, all components were added up to get the total VFA.

Methane content in assays was determined by using a gas chromatograph (Perkin-Elmer

Arnel Clarus 500) equipped flame-ionization detector (FID) and Alumina column (30 m x 0.53 mm) as described by Bayr et al. (2013). A special gas tight syringe (VICI Pressure-Lok® Precision Analytical Syringe) with removable needle was used for sampling 0.1 ml of sample taken at a time and injected directly into the chromatographic column. The methane measurement was done everyday during the first week of anaerobic digestion, and then every 2-3 days until the end of biogas production. The methane potential of the pretreated materials was calculated as methane yield per $VS_{original}$ (VS added before the pretreatment). The methane yields were expressed as VS (ml CH_4 per $VS_{original}$) and fresh material (ml CH_4 per $FM_{original}$) at normal conditions (T= 273 K, p= 1.013 bar). The summary of the steps used in this particular study is presented in graphically as shown below (Figure 9).
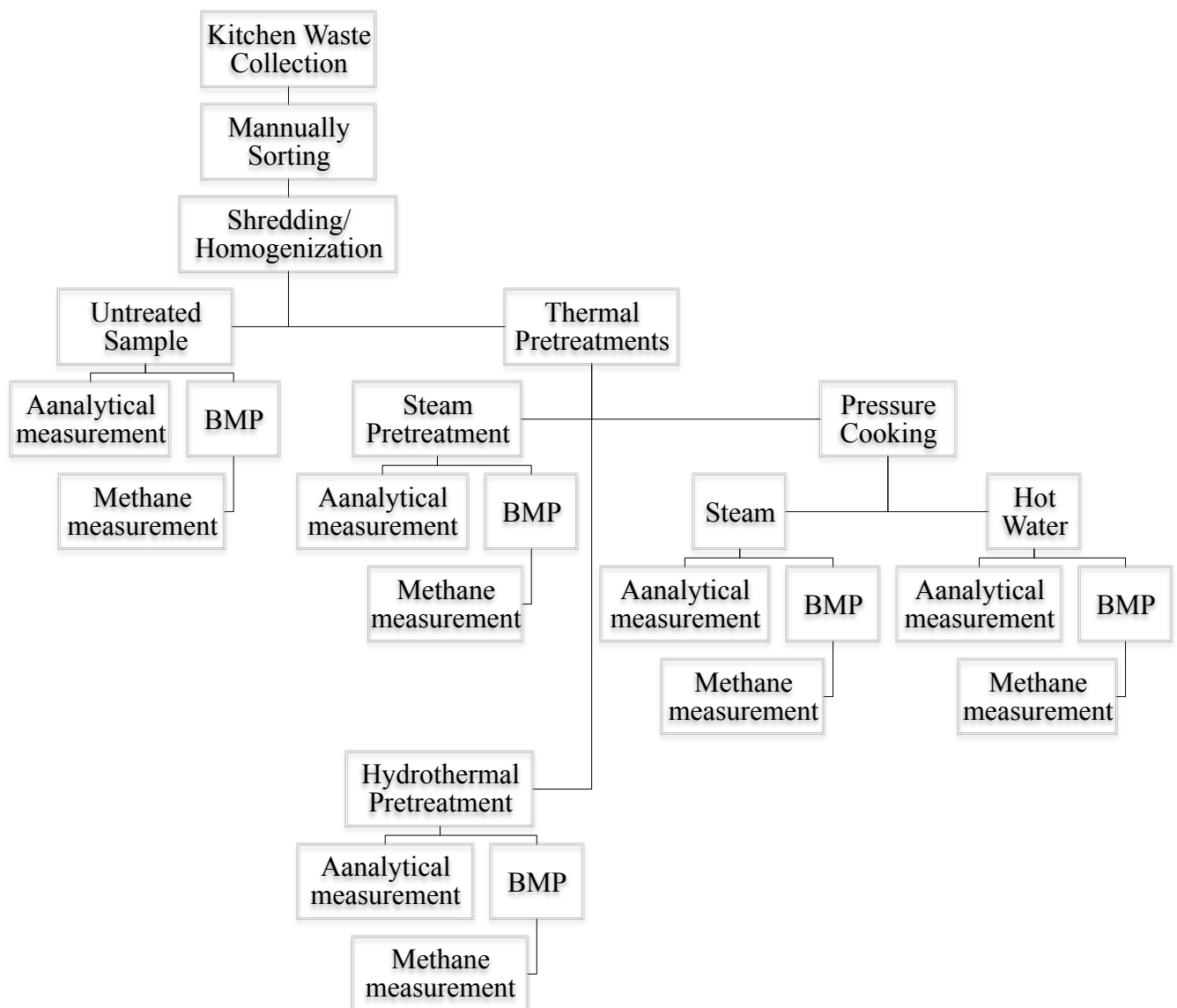

Figure 9. Summary of the steps and processes involved in this study (BMP: biochemical methane potential test).

3 RESULTS

3.1 Kitchen waste composition

Results showed that kitchen waste used in the present study mainly contain vegetables trimmings (56%), fruit skins and spoilt fruit (43%) and bread (1%). Eggshells, teabags, coffee grounds and paper napkins were the minor components. Figure 10 presents the main composition of the studied kitchen waste.
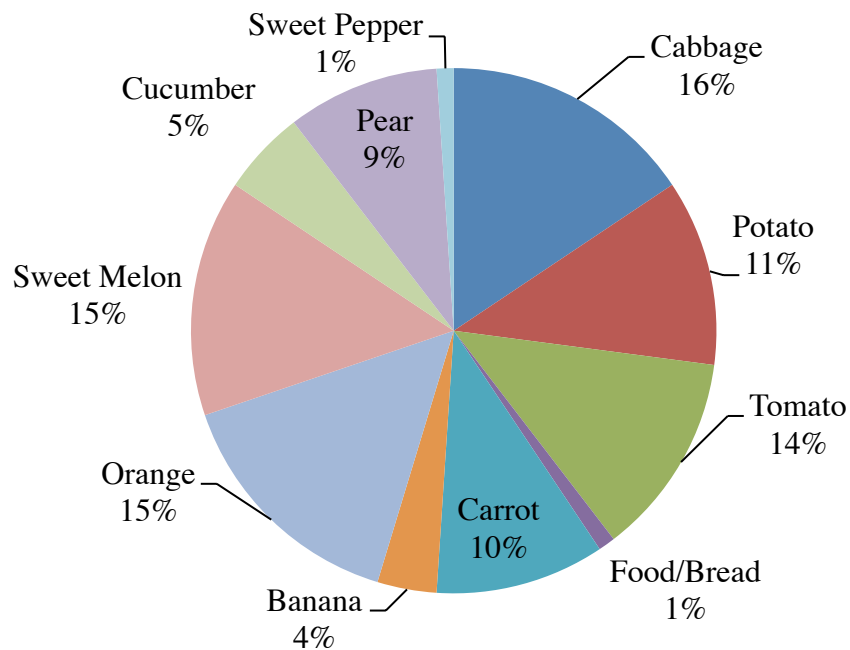

Figure 10. Composition of kitchen waste.

However, some of the materials were lost during pretreatment process even though care and caution were taken during the experiments. The losses were the combination of evaporation and handling losses as it presented in Table 3 below.

Table 3. Mass balance from thermal pretreatment process.

Pretreatment Methods	Before pretreatment			After pretreatment		
	Sample (g)	Water (ml)	Total mass (g)	Evaporation loss (ml)	Handling loss (g)	Total mass (g)
PC1	70	210	280	21	5	254
PC2	70	210	280	11	5	264
Steam	70	150	220	43	5	172
Hydrothermal	70	210	280	0	4	276

Note: PC1: Pressure-cooking with steam, PC2: Pressure-cooking with water.

3.2 Chemical characteristics of kitchen waste before and after pretreatments

The chemical characteristics of untreated and pretreated kitchen waste are summarized in Table 4. Since water was added during thermal pretreatment process, the concentrations of chemical composition of the material per unit volume were decreased. Therefore, all the chemical compositions of pretreated kitchen waste such as TS, VS, TKN, NH_4-N, SCOD and VFA were multiplied with the dilution factor in order to find the effect of thermal pretreatment on those chemical compositions.

The result shows that untreated kitchen waste had TS and VS content of 12.6 % and 11.9 %, respectively. Kitchen waste subjected to PC1, PC2 and steam pretreatment resulted in an increase in TS and VS. On the other hand, TS and VS content of hydrothermally pretreated kitchen waste remained more or less unchanged.

All pretreatments resulted in an increase in SCOD, TKN and NH_4-N, but completely loss of NH_4-N was noticed from kitchen waste subjected to PC2. Losses of TVFA were also noticed from all pretreated samples. The decrease in TVFA concentration was high in samples subjected to steam pretreatment followed by PC2, hydrothermal and PC1. The measured TVFA values in mg/l were converted to mgSCOD/l by multiplying with conversion factors as it mentioned in chapter two of this study.

Table 4. Chemical characteristics of untreated and pretreated kitchen waste.

Samples	pH	TS (%)	VS (%)	TKN (g/l)	NH_4-N (mg/l)	SCOD (g/l)	TVFA (mgSCOD/l)
Untreated kitchen waste	4.69	12.6	11.9	0.63	4.10	80.3	1108
Pretreated kitchen waste							
PC1	4.65	13.3	12.6	1.09	5.50	85.5	707
PC2	4.63	13.2	12.4	4.90	0.00	89.3	355
Steam	4.63	15.1	14.4	1.60	4.50	90.7	200
Hydrothermal	4.50	12.8	12.0	2.10	70.1	100.6	597

Note: PC1: Pressure-cooking with steam, PC2: Pressure-cooking with water.

The percentage changes in TS (1.6-20.2 %), VS (0.8-21.2 %), VFA (-41.5 % to 85.7 %) and SCOD (6.4-25.2 %) with respect to untreated kitchen waste were presented on Figure 11.
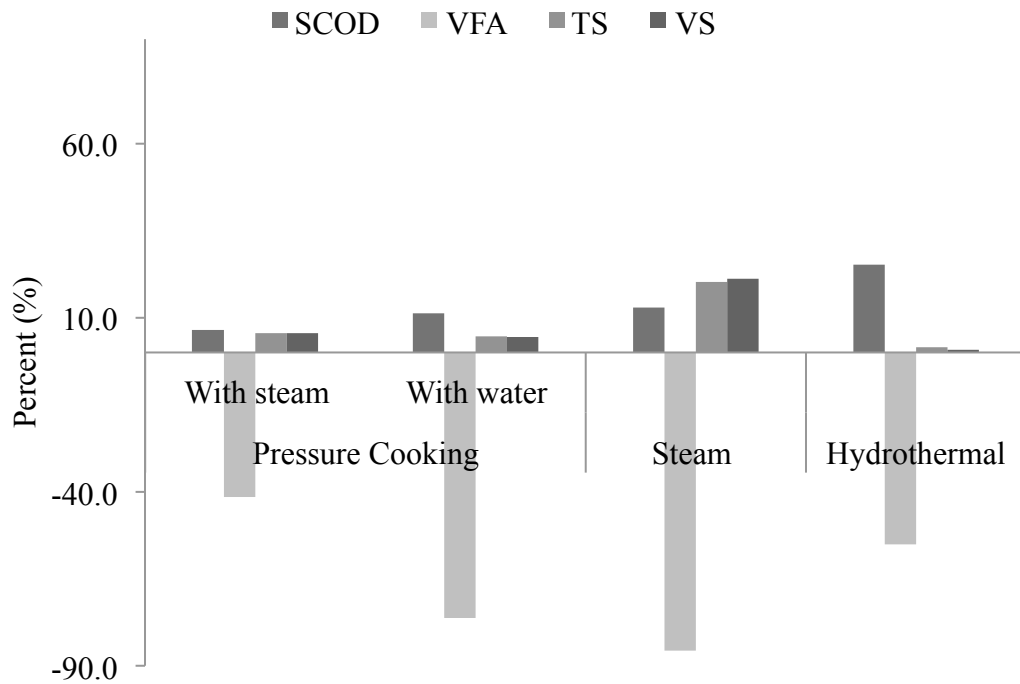
Figure 11. Percentage change in TS, VS, VFA and SCOD with respect to untreated kitchen waste.

3.3 Batch experiment

As it presented graphically (Figure 12), methane production at 35 °C was delayed by 4 to 5 days but most of the biogas production was completed within 44 days of incubation. On the other hand, methane production started immediately in most of the assays at 55 °C but took 63 days to realize the potential. Approximately 90 % of the total methane was produced in the last 23 and 37 days of digestion at 35 °C and 55 °C, respectively.

The cumulative methane yields obtained from untreated and pretreated kitchen waste were presented in ml (Figure 12) and ml/gVS (Table 5). As it shown on Figure 12, the cumulative methane yields obtained from untreated kitchen waste were 532 ml at 35 °C and 547 ml at 55 °C. Similarly, the cumulative methane yields from pretreated kitchen waste were varied from 512-546 ml at 35 °C and 520-558 ml at 55 °C in which the maximum yields were noticed from kitchen waste subjected to steam pretreatment (546 ml) at 35 °C and PC1 (558 ml) at 55 °C.
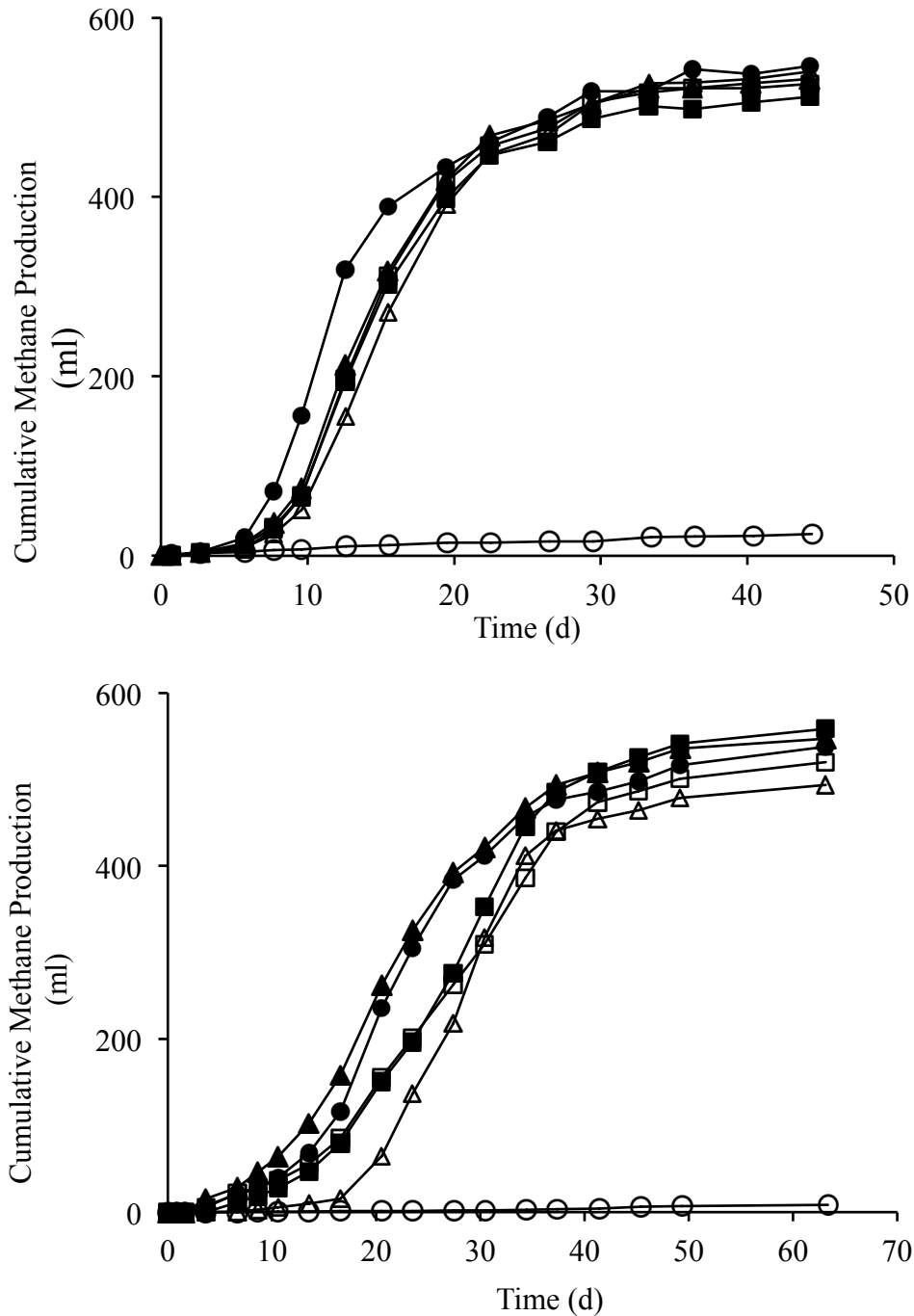
Figure 12. Cumulative methane yields obtained during anaerobic digestion of kitchen waste in batches at 35 °C (above) and 55 °C (below): untreated kitchen waste (▲), PC1 (■), PC2 (Δ), steam pretreatment (●), hydrothermal pretreatment (□) and inoculum/control (o). Note: (PC1: Pressure-cooking with steam, PC2: Pressure-cooking with water).

As it mentioned earlier, the difference between methane yields among pretreated and untreated samples at 35 °C and 55 °C in ml/gVS and ml/gFM were presented on Table 5. In addition, the percentage change of methane yield, which varied from -4 to 3 % at 35 °C and from -10 to 2 % at 55 °C with respect to untreated kitchen waste, was also presented in the same table below.

Table 5. Mean methane yields obtained during anaerobic digestion of kitchen waste inbatches at 35 °C and 55 °C (± standard error).

Kitchen Waste	Mesophilic (35 °C)			Thermophilic (55 °C)		
	ml/gVS	% (+/-)	ml/gFM	ml/gVS	% (+/-)	ml/gFM
Untreated	426±14	0	51±2	452±26	0	54±3
Pretreated						
PC1	410±13	-3.8	49±2	462±17	2.2	55±2
PC2	434±10	1.9	52±1	406±2	-10.2	48±0.2
Steam	439±1	3.1	53±0.1	445±29	-1.6	53±0.3
Hydrothermal	422±9	-0.9	50±1	430±5	-4.9	51±0.6

Note: +/-: Percentage increase/decrease, PC1: Pressure-cooking with steam, PC2: Pressure-cooking with water).

Among the pretreatments, kitchen waste subjected to steam pretreatment at 35 °C and PC1 at 55 °C had relatively the highest methane yields. On the other hand, the lowest methane yields were obtained from PC1 at 35 °C and PC2 at 55 °C. In general, methane yields obtained at 55 °C were higher than those obtained at 35 °C except for kitchen waste subjected to PC2. Among all pretreatments, kitchen waste subjected to PC1 had the highest methane yield at 55 °C (462 ml/gVS).

4 DISCUSSION

4.1 Effect of thermal pretreatment on chemical characteristics of kitchen waste

The results in the present study showed that the studied thermal pretreatments affected the chemical characteristics of kitchen waste. Compared to untreated kitchen waste, TS and VS of thermally pretreated kitchen waste were increased by 1.6 to 20.2 % and 0.8 to 21.1 %, respectively. This result was compared with two previous studies conducted by Ma et al. (2011) and Dlabaja & Malaťák (2013). Ma et al. (2011) reported that TS and VS of thermally pretreated kitchen waste at 120 °C for 30 minutes were increased by 5.4 and 4.5 %, respectively. Similarly, Dlabaja & Malaťák (2013) also noticed that TS value was increased by 4.9 % after mechanically crushed kitchen waste was thermally pretreated at 70 °C for 70 minutes. The observable difference between the reported results and the present study might be due to the difference in the chemical composition of the materials used and pretreatment conditions (temperature and holding time). The possible reason for high TS and VS content of pretreated kitchen waste is due to loss of water via evaporation during cooking and cooling process (Das et al. 2006). This is evident from the TS and VS contents of the hydrothermally pretreated kitchen waste of this study (Table 3). It is clearly seen that water loss due to evaporation during hydrothermal pretreatment was almost negligible, and as a result the change in TS and VS was more or less unchanged.

The low TKN and NH_4-N content in the untreated kitchen waste was obviously due to low nitrogen content of the original sample, which mainly contains fruits and vegetables. However, all thermal pretreatment techniques were increased the TKN and NH_4-N concentration, except that of PC2. The increase in TKN and NH_4-N might be due to the degradation of protein molecule (Athanasoulia et al. 2007). The losses of most volatile fatty acids during pretreatment process might be due to high temperature used for the pretreatments. On the other hand, the increase in SCOD concentration by 6.4-25.2 % after thermal pretreatment was due to the conversion of organic substance in kitchen waste into soluble matter (Zhou et al. 2012). These results are in accord to the previous results reported by Ma et al (2010), Marin & Kennedy (2010) and Liu et al. (2012). For instance, the increase in SCOD content of 25 % for the hydrothermally pretreated kitchen waste in the present study was close to the value of 24 % reported by Ma et al.

(2011). This was probably due to more or less similarity of pretreatment conditions i.e. temperature and residence time. The highest SCOD noticed from hydrothermal and steam pretreatment was due to the longer pretreatment time. These results are in agreement to Liu et al. (2012), Xiao and Liu (2006) who suggested that long the pretreatment time release more intracellular matter and polymers of the substrate into water, which will increase the SCOD concentration of the sample.

4.2 Effect of thermal pretreatment on methane yield of kitchen waste

The mean methane yield of 426 ml/gVS obtained from untreated kitchen waste at 35 °C in the present study was more or less similar to the values reported in the literature for food waste, fruit and vegetable, restaurant waste, commercial kitchen waste (Heo et al. 2004, Banks et al. 2008, Neves et al. 2008, Chen et al. 2010). On the other hand, methane yield of 452 ml/gVS obtained from untreated kitchen waste at 55 °C in the present study was higher than the yields reported by Zhang et al. (2007) and Banks et al. (2008), who found 435 ml/gVS at 55 °C and 388 ml/gVS at 56 °C, respectively. This might be due to difference in chemical characteristics and composition of the feedstock. Cho et al. (1995) have showed this difference by analyzing the methane potentials of different kitchen waste composition. They found that the methane potential of cooked meat, boiled rice, fresh cabbage and mixed food wastes varied from 277 to 482 ml/gVS after 28 days of digestion at 37 °C due to difference in chemical components such as lipids, starch and cellulose (Zaman 2010).

However, the results showed that the studied thermal pretreatment techniques were less effective in improving the methane yields of kitchen waste. The maximum increase in methane yields was 3.1 % at 35 °C and 2.2 % at 55 °C and was noticed with steam pretreatment and PC1, respectively. However, few other thermal pretreatment techniques resulted in low methane production rates and yields at mesophilic and thermophilic incubation temperatures.

4.2.1 Difference between the three thermal pretreatment techniques

The high variation in methane yields for thermally pretreated kitchen waste i.e. form 410-439 ml/gVS at 35 °C and 406-462 ml/gVS at 55 °C clearly indicates that the effect of thermal pretreatment on methane yield vary with respect to pretreatment temperature, residence time and treatment techniques (Kumar et al. 2006).

The increase in methane yields by 1.9 to 3.1 % when kitchen waste was subjected to PC1 at 55 °C, PC2 at 35 °C and steam pretreatment at 35 °C were in agreement to the results reported by Ma et al. (2011) and Ariunbaatar (2013). On the other hand, the low or decrease in methane yields by 0.9-10.2 % from kitchen waste subjected to PC1 at 35 °C, PC2 at 55 °C, hydrothermal pretreatment at both temperatures (35 °C and 55 °C) and steam pretreatment at 55 °C were similar to the studies reported by Dlabaja & Malatak (2013) and Liu et al. (2012). The decrease in methane yields might be due the loss of VFA during thermal pretreatment, the possible formation of inhibitory products and loss of volatile organic materials (Ariunbaatar 2013, Chen et al. 2010, Hendriks & Zeeman 2009). Basically, the possibility of inhibitors formation during steam and water pretreatment is relatively high (Hendriks & Zeeman 2009). Marin and Kennedy (2010) demonstrated this by analyzing the methane potential of microwave irradiation pretreated kitchen waste (i.e. water and steam haven't used for thermal pretreatment) and noticed 40 % improvement in methane yields in the pretreated biomass.

4.2.2 Difference between mesophilic and thermophilic temperatures

The difference between methane production rates and yields noticed at 55 °C than at 35 °C in the present study was obviously due to difference in anaerobic inocula used for digestion process and/or due to further degradation of VFA in case of thermophilic digestion (Banks et al. 2008). These results were in accord to those reported by Chen et al. (2010) and Banks et al. (2008). Furthermore, previous studies revealed that thermophilc bacteria have higher growth rate than mesophilic and therefore they can consume higher amount of organic matter (Vindis et al. 2009), i.e. thermophilc bacteria have high organic removal and degradation rate than mesophilc bacteria (Chen 2010). However, some thermal pretreatment techniques were better at 35 °C than 55 °C and vice versa, which might be due to the differences in ability to tolerate inhibitors or the way they response to environmental changes (Yilmaz et al. 2008).

Methane production rates in this study both at 35 °C and 55 °C, in general, didn't stop to obey sigmoid function (S curve) (Figure 11). It was observed that the biogas production was slow at the beginning of digestion due to slow microbial growth (Abubakar & Ismail 2012), and after slow start, biogas production increase rapidly before it reaches final biogas yield. This might be due to the possible recovery (Chen et al. 2010) and rapid growth of methanogens (Abubakar & Ismail 2012). From this, it is clear that there

is a direct relationship between methanogenic bacteria growth and biogas production rate (Abubakar & Ismail 2012).

Generally, the effect of thermal pretreatment on this study was not significant especially on methane yield because the studied kitchen waste contains highly biodegradable materials. However, it's obvious that if this technology applied to lignocellulosic materials under optimal conditions (temperature and residence time) it will improve the biodegradability of the materials and increase the conversation efficiency of anaerobic digestion. Thermal pretreatment not only improve the methane yield of the substrate, but also sanitize the material if waste materials such as kitchen waste or organic fraction of municipal solid waste (OFMSW) is considered. In addition, the negative impact of this technology to the environment is relatively low, because it only requires water and no need of chemicals or external solvent (Triantafyllidis et al. 2013).

However, thermal pretreatment requires energy, which is the main factor affecting the net profits of the whole process if cost of the pretreatment is considered. According to previous studies, thermal pretreatment of kitchen waste was economically feasible even though it depends on the type substrate, pretreatment conditions (temperature and time) and method used for pretreatment (Ma et al. 2011). Sometimes, profits can also be achieved without pretreatment depend on the material, and it's recommended to evaluate the required energy and the energy content of the final product especially at full-scale application. Therefore, these technologies still require further research to check the energy balance of the whole process and to investigate the optimal temperature and residence time of thermal pretreatment for maximum methane yield.

5 CONCLUSTIONS

The present study was conducted to evaluate the effect of thermal pretreatment on chemical characteristics and methane yield of kitchen waste. Various chemical characteristics such as pH, TS, VS, SCOD, VFA, NH_4-N and TKN were measured and analyzed to compare the effect of pressure cooking, steam and hydrothermal pretreatment on them.

The results showed that the pH of untreated and pretreated kitchen waste was more or less similar. After thermal pretreatment, the TS and VS of the studied material increased by 1.6-20.2 % and 0.8-21.1 %, respectively. Thermal pretreatments viz., pressure-cooking, steam and hydrothermal pretreatment could improve the SCOD, NH_4-N and TKN concentration in the pretreated kitchen waste but could result in loss of VFA (41.5-85.7%). However, complete loss of ammonium nitrogen was noticed in PC2.

Among the tested pretreatments, PC2 and steam pretreatment resulted in relatively higher methane yields than untreated kitchen waste at 35 °C. At 55 °C, only PC1 resulted in higher methane yields than untreated kitchen waste. Methane yields obtained at 55 °C were in general higher than those obtained at 35 °C.

Generally, the results of this study indicate that all the studied thermal pretreatment techniques (PC1, PC2, steam and hydrothermal pretreatments) had affected the chemical characteristics of kitchen waste to a large extent but no or little effect on methane yields.

ACKNOWLEGMENTS

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APPENDICES

Appendix A: Equations

Dilution Factor (DF) $= \dfrac{Final\ Volume/Mass}{Aliquot\ Volume/Mass}$ (1)

Where, Final Volume = Aliquot + Diluent

Total Solid (TS)

$$Total\ Solids\ (TS), \% = \frac{(A - B)}{(D - B)}\ x\ 100\%$$ (2)

Where, *A* weight of dish + dry sample (in grams), *B* weight of dish (in grams) and *D* weight of dish + wet sample (in grams)

Volatile Solid (VS)

$$Volatile\ Solids\ (VS), \% = \frac{(A-C)}{(A-B)}\ x\ 100\%$$ (3)

Where, *A* weight of dish + dry sample (in grams), *B* weight of dish (in grams), *C* weight of dish + sample after ignition (in grams)

Ammonia Nitrogen (NH4-N)

$$NH4 - N = \frac{((A - B)\ x\ 280)}{ml\ sample}$$ (4)

Where, NH_4-N ammonia nitrogen (mg/l), *A* volume of sulfuric acid titrated for sample (ml), *B* volume of sulfuric acid titrated for blank (ml)

Total Kjeldahl Nitrogen (TKN)

$$TKN = Organic\ N + Ammonia\ N$$ (5)

Soluble Chemical Oxygen Demand (SCOD)

$$CODcr = [8000\ x\ Cfe\ x\ ((V3 - V4)/V5)x\ dilution]$$ (6)
$$Cfe = \ 0.24\ x\ V1/V2$$

Where, *Cfe* Iron II solution, mol/l, COD_{cr} chemical oxygen demand, mg/l, *V1* amount of K_2CrO_7 = 1ml, *V2* amount of titrated iron (II) solution (ml), *V3* amount of blank (ml), *V4* sample volume in titration (ml), *V5* volume of sample used = 2 ml

Volatile Fatty Acid (VFA)

Total VFA (mg/l) = Acetic acid (mg/l) + Propionic acid (mg/l) + Isobutyric acid

(mg/l) + Butyric acid (mg/l) + Isovaleric acid (mg/l) + Valer-

ic acid (mg/l) + Caproic acid (mg/l) (7)

Total VFA (mgSCOD/l) = 1.066 x acetic acid (mgSCOD/l) + 1.514 x propionic

acid (mgSCOD/l) + 1.818 (butyric + iso-butyric acid)

(mgSCOD/l) + 2.039 (valeric + iso-valeric + caprioc

acid) (mgSCOD/l)

Methane Yield

$$CH_4 \text{ (ml)} = \frac{\text{sample Area [uV*sec] * standard gas (\%)*head space (ml)}}{\text{standard gas Area [uV*sec]}}$$

$$CH4 \text{ (ml/d)} = \frac{CH4 \text{ (ml)}}{Number\ of\ days\ (d)} \tag{8}$$

$$CH_4/g \text{ VS added} = \frac{CH4\ from\ mixture\ (ml) - CH4\ from\ Inoculum\ (ml)}{gVS\ added}$$

Appendix B: Experimental procedures

TS and VS Measurement

- Prepare evaporating dishes for each sample by cleaning and ignition at 500 °C for 1.5 to 2 hours in a muffle furnace
- Cool, desiccate, weigh, and store dish in a desiccator until ready for use
- Transfer sample to the prepared dish and weigh (weight of wet sample plus dish) and evaporate to dryness in a drying oven for at least 16 hours at 98 to 105 °C
- Cool dish in the desiccator and weigh (weight of dry solids)
- Calculate the TS according to the above equation (1)
- After completing the total solids analysis, ignite the sample and dish for 1.5 to 2 hours at 550 °C in a muffle furnace
- When cooling dish in the desiccator, open the top cover for about 2 min to let off the hot gas and close desiccator to complete cooling
- Weigh the cooled dish
- Calculate the VS according to the above equation (2)

TKN Measurement

Preparing the tubes

- Add suitable amount of sample into the tube
- Dilute it with distilled water up to 50ml
- Add:
    - 1 tablet of K_2SO_4 + $CuSO_4$
    - 1 boiling stone
    - 10 ml H_2SO_4
- Wait for 30 minutes
- Put the heating equipment on and set the temperature to 200 (with proper adjusting depending on the heating equipment used, detailed instruction can be seen near the heating equipment)
- Put the tubes on heating equipment (after it reached the set temperature)
- Carefully put the top on the tubes and turn water cooling on
- Heat the tubes at 200 for 1 hour
- Set the temperature to 370 and heat the tubes for 1 hour
- Take the tubes from equipment and let them cool with the water cooling on

Distillation and titration similar to NH_4-N, with few exceptions

- Dilute the samples to 75ml in the tubes before distillation
- Distillation is done in the equipment near the windows
- No pH adjustment or buffer before distillation
- Add NaOH by pulling the handle in the distillation equipment, before starting the distillation, the content in the tube should turn into black

This distillation equipment has to be rinsed after setting the power of, rinse once with tap water and once with distilled water.

NH$_4$-N Measurement

General

- Analyze the sample on the sampling day unless store for a week by setting the pH of the sample below 2 with 4M H$_2$SO$_4$
- Calibrate the pH meter before use
- Dilute the sample with the appropriate dilution factor
- Measure each sample at least two times

Distillation of the sample

- Put 25 ml of boric acid (4 %) into the Erlenmeyer-flask
- Put 5ml of buffer into a tube, measure the sample
- Check that the pH in the tube in the tube is at least 9.5, if not add some 1M NaOH (few drops is enough)
- Put the tube on the left handle side and the Erlenmeyer-flask on the right hand side on white cork
- Steam on
- Distillate until there is 125 ml liquid in the Erlenmeyer-flask
- Take the white cork off
- Steam off
- Take the tube off
- Take the Erlenmeyer-flask off

Titration of the sample

- Titrate the sample (now in the Erlenmeyer-flask) with 0.01 M H$_2$SO$_4$ until the pH is 5.1
- Note the titrated amount from the automatic burette

VFA Measurement

Preparing samples

- Add 0.5 ml of oxalic acid in each eppendorf tube in order to set the pH below 2 to get all the acids in dissociated from (-COOH)
- Centrifuge the sample for 10 min in 12000 rpm because sample may contain solid matter or sample may form solids when oxalic acid is added
- Transfer 1 ml of supernatant to another eppendorf tube
- Dilute the sample, especially sample containing high VFA concentration to get a signal within the calibration curve. REMEMBER: 1 ml of sample + 0.5 ml of oxalic acid = 1.5 times diluted! Dilution depends on soluble COD of the sample in the following way:

    < 2 g COD 1.5 times diluted

    2-4 g COD 3 times diluted

    4-8 g COD 6 times diluted

    8-10 g COD 7.5 times diluted

    10-20 g COD 15 (or 10) times diluted

    20-30 g COD 20-30 times diluted

These are suggestive values; it's usually not necessary to dilute this much. The dilution factor may vary depending sample characteristics. In anaerobic digestion, feedstock VFA levels vary greatly, and digested samples usually requires dilution of 1.5-3, not depending on SCOD. If the sample was not diluted enough, results are reliable at least up to 5 times over quantitation limits. Proper dilution is preferred due to increased liner life.

Dilution	Sample (μl)	Ox (μl)	H_2O (μl)
1.5	1000	500	0
3	500	500	500
6	250	500	750
7.5	200	500	800
10	150	500	850
15	100	500	900
20	75	500	925
30	50	500	950

Analysis

- Rinse the syringe, washing vials and waste vials regularly with acetone (depending on samples, other solvents, e.g. MTBE, maybe more efficient)
- Change spetum every 100th injection
- If chromatograph has been out of use for longer time (e.g. over a month), do some water standard runs before calibration
- Put a water run first and last on the sequence and between appropriate amount of sample runs

SCOD Measurement

Preparation

- Centrifuge the sample at 3000 rpm for 10 to 15 minutes and then filter through a 1.6μm microfibres filter with water suction
- Dilute the supernatant sample with appropriate dilution factor, especially if the sample contains high organic matter
- Put the SCOD reactor (the boiler) on. When the heating light switches off, the reactor is at 150 °C
- Pump the automatic pipettes a few times with the tap closed to remove air bubbles

Analysis

Control tubes

- Prepare two control tubes
- Pipette 1 ml of potassium dichromate ($K_2Cr_2O_7$), 2 ml of distilled water and 3 ml of strong sulphuric acid (H_2SO_4) into both tubes

Zero samples

- Prepare two zero tubes
- Pipette 1 ml of potassium dichromate ($K_2Cr_2O_7$), 3 ml of sulphuric acid/silver sulphate (Ag_2SO_4/H_2SO_4), 200 μl of mercury sulphate ($HgSO_4$), and 2 ml of distilled water into both tubes

Sample tubes

- Prepare two tubes for SCOD samples
- Pipette 2 ml of sample, 1 ml of potassium dichromate ($K_2Cr_2O_7$), 3 ml of sulphuric acid/silver sulphate (Ag_2SO_4/H_2SO_4), 200 μl of mercury sulphate ($HgSO_4$) into both tubes. If the sample tube turns green after adding the sample into the tube, the sample contains too much organic material and has to be dilute
- After preparation, close the caps of the tubes tightly, shake the tubes well and put the zero and the sample tubes (not control) into the heated SCOD reactor
- Turn the clock to 120 min and switch the timer button on. The reactor will be switched off automatically after two hours.

Titration

- All tubes (controls, zeros and samples) need to be titrated

- After boiling, take off the tubes from the reactor and let to cool down to room temperature

- Take one tube at a time, open it and add two drops of indicator solution (ferroine) into the tube

- Drop a little magnet into the tube and place it on the magnetic stirrer and titrate with iron (II) solution

- While titrating, the color of the solution in the tube first changes into bluish green and then very quickly into red

- Stop titrating at this point and write down the amount of iron (II) solution used for the titration (from the byrette)

- Finally calculate the SCOD of the sample by equation 5 above

CH$_4$ Measurement

Preparing standard gas (30 % CH$_4$ and 70 % N$_2$)

- Push needles through the standard bottle and attach gas tubes to the standard bottle and bubble bottle

- Open main valve, needle valve and regulator valve and bubble for 3 to 5 min to the standard bottle

- When its ready, close all the opened valves and remove needles from the standard bottle

FID measurement

- Login to the computer and start chromatography data system: totalchrom navigator (TCNav) software

- Open air and hydrogen valves

- Ignite FID and wait until GC says 'ready'. It might take a while the FID to ignite (wait or try again)

- Inject 0.1 ml of standard gas to the FID column by air thigh syringe at least three times

- Then, inject at least two samples (sample volume 0.1 ml) to the FID column

- After all injections are done and all the peaks have appeared to real time plot, stop run

- Finally close air and hydrogen valves and do the calculation according to the above equation (7)