Enhanced Anaerobic Biogasification from Rice Straw Pretreated by Hydrothermal Technology

A Dissertation Submitted to
The Graduate School of Life and Environmental Sciences,
The University of Tsukuba
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy in Environmental Studies
(Doctoral Program in Sustainable Environmental Studies)

Leilei HE

Abstract

Rice straw is an abundant agricultural waste with annual global quantity of 685 million tons. With the increasing need of crop production and much dependence on fossil fuels, more and more crop straws are burned or discarded in the field due to lack of cost-effective treatment and recycling methods, triggering severe environmental problems. Anaerobic digestion is a prospective alternative for sustainable utilization of rice straw. The fermentation of rice straw, however, is very difficult in practice due to its lignocellulosic nature and high content of lignin. Thus, in order to improve degradability of rice straw for anaerobic digestion, pretreatment is prerequisite for a high yield of biogas. Hydrothermal treatment (HTT), an environment-friendly process, can be a prospective alternative for the pretreatment of rice straw resulting in efficient hydrolysis of the organic matter contained. Still, up to now no detailed information could be found with respect to HTT pretreatment for hydrogen production and subsequent methane production from rice straw.

In this study, in order to utilize rice straw effectively for anaerobic digestion, HTT was used as pretreatment for enhanced biogasification. The feasibility of biohydrogen production from the hydrothermal pretreated rice straw substrate was investigated. Furthermore, the feasibility of methane fermentation from hydrogen fermentation effluent was also discussed.

In the preliminary experiments, the effect of different HTT conditions, including temperature (190-290℃), holding time (1.5 min) and solid content (4-20%) on rice straw pretreatment were investigated. In addition, batch experiments of mesophilic anaerobic digestion were also carried out to verify the enhancement on biogasification when pretreated rice straw by HTT was used as feedstock. The results showed that higher temperature was effective for reduction of total solids (TS) and volatile solids (VS). However, when temperature was above 230℃, soluble chemical oxygen demand (SCOD) did not significantly increase with the increase of temperature. Moreover, the released soluble products were also detected. Less soluble carbohydrates (0.1-2.9 mg/g-VS) were detected at higher temperatures

(＞230℃). When considering high hydrogen production and energy recovery, moderate temperature (210℃) and high solid content (20%) in HTT was found to be favorable for subsequent biogasification.

The followed-up experiments tested the effect of determined peak temperature (150℃ and 210℃, i.e. HTT150 and HTT210, respectively) and holding time (0-30 min) on the solubilization of rice straw at TS of 20% and then subsequent H_2 production from the resultant substrates. No obvious degradation was detected in lignin content under all tested HTT conditions which did open up the surface structure and have efficient solubilization effect on rice straw. Soluble carbohydrates produced from straws during HTT210 was found to have strongly (r=0.9987) positive correlation with the subsequent H_2 yield. The maximum soluble carbohydrates, 80 mg/g-VS was achieved at HTT210 for 0 min of holding correspondingly to the highest hydrogen yield (28 ml/g-VS), about 93-fold higher than the control, suggesting holding time is crucial for HTT pretreatment combined with subsequent H_2 production.

In addition, the effluent from hydrogen fermentation of HTT pretreated rice straw substrate was used for methane fermentation. The feasibility of two-stage anaerobic digestion (i.e. using the first stage for H_2 production and the second stage for CH_4 production, resepctively) was explored. And conventional single-stage anaerobic digestion was also conducted for performance evaluation and comparison between the single- and two-stage processes. The two-stage anaerobic digestion showed a higher methane production potential than the conventional single-stage digestion. Compared to the single-stage process, the whole fermentation period of the two-stage process was shortened from 45 to 31 days. In the single-stage process, compared to the control reactors HTT210 pretreatment seems to have negative effect on methane production potential with corresponding methane yields varied from 31 to 76 ml/g-VS. While the HTT150 pretreated straw showed higher methane production potential than HTT210 pretreated straw in both the single- and two-stage processes. In addition, the maximum methane yield, 162 ml/g-VS was obtained from rice straw after HTT150 for 20 min in the methane producing stage of the two-stage anaerobic digestion.

The results from this study is believed to provide important information (mainly including temperature and holding time which will greatly influence the reactor design and energy input) on HTT pretreatment of rice straw for anaerobic biogasification. This work is also useful and important for the combined application of HTT pretreatment with fast biohydrogen fermentation technique of crop straws. Also, the results imply that it's feasible to use HTT as a pretreatment method of rice straw for subsequent H_2/CH_4 production in practice. In addition, the two-stage digestion process seems to be more promising than the single-stage method for further enhancement of biogasification from HTT pretreated rice straw substrate.

Keywords: Rice straw; Hydrothermal treatment; Hydrolysis; Single-stage anaerobic digestion; Two-stage anaerobic digestion

Contents

List of Tables

List of Figures

1. Introduction

1.1. Lignocellulosic biomass and its utilization for energy production

The global energy demand was reported to expand 2.4-fold from 1971 to 2010 (about 11,700 million tons of oil equivalent in 2010), especially the energy demand of Asian region which increased rapidly over recent years and accounted for 70% of the increment in global energy consumption from 2000 (Matsuo et al., 2013). Nowadays, most of global energy demand relies on fossil fuels, including oil, coal and natural gas, which was estimated to share 78.4% of global energy consumption accounted in 2012 (REN21, 2014). Namely, fossil fuels are the major energy resources. However, fossil fuels are non-renewable energy resources, which would bring about energy crisis and environmental problems (Lin and Chen, 2006). In order to minimize their environmental pollutions and meet the increasing energy demand, an urgent need is to seek alternative energy resources for the global sustainable development, among which renewable energy resources are the most prospective candidates. Therefore, development of technologies for renewable energy conservation is the most challenging subject in the 21st century.

Biogas, one of the most promising renewable energy sources, is regarded as a substitute for fossil fuels to reduce greenhouse gases (GHGs) emission. The biogas mainly composed of methane (CH_4) and carbon dioxide (CO_2) is produced generally from biomass by anaerobic digestion. Thus, anaerobic digestion, as a cost-effective and environment-friendly waste treatment method, will play a vital role in meeting the global ever-increasing energy requirements in the future. In addition, anaerobic digestion is applicable to a wide range of biomass.

Biomass can be classified into five categories including virgin wood, energy crops, agricultural residues, food waste, industrial waste and their co-products. A wide range of biomass has been considered as potential sources for methane production (Nallathambi Gunaseelan, 1997). Among them, lignocellulosic biomass is abundant with an annual production of about 200 billion tons worldwide (Ren et al., 2009). Being derived from biological origin, lignocellulosic biomass contains relatively high contents of lignin, hemicellulose, cellulose, and pectin, forming a molecular matrix with some low contents of monosaccharides, starch, protein, or oils (Wu et al., 2010). Due to containing a large amount of cellulose and hemicellulose, lignocellulosic biomass can be used for biogas production through fermentation.

Rice straw is an abundant lignocellulosic waste with annual global quantity of 685.24 million tons (Lim et al., 2012). The annual productions of rice straw in Asia, Americas, Africa, Europe and Oceania are estimated to be 618.24, 38.10, 24.51, 4.10 and 0.29 million tons, respectively (FAOSTAT, 2009). With the increasing need of crop production and much dependence on fossil fuels, more and more crop straws are burned or discarded in the field due to lack of cost-effective treatment and recycling methods, triggering severe environmental problems. According to the report by UNEP (2009), open field burning carried out after harvesting season is perhaps the most common practice of handling rice straw in many countries in Asia. Anaerobic digestion is a prospective alternative for the sustainable utilization of rice straw.

1.2. Importance of pretreatment for anaerobic digestion of lignocellulosic biomass

The fermentation of rice straw, however, is very difficult in practice due to its lignocellulosic nature and high content of lignin, a major barrier for lignocellulosic biomass in the bioconversion process. Thus, in order to improve degradability of lignocellulose for anaerobic digestion, pretreatment of rice straw is prerequisite for a high yield of biogas (Zheng et al., 2014).

Pretreatment can improve hydrolysis of lignocellulosic biomass, thus enhance methane production in the subsequent anaerobic digestion. Compared to conventional anaerobic digestion (without pretreatment) which needs long digestion time and large digester, pretreatment can shorten digestion time and reduce reactor volume due to the enhanced hydrolysis. It is meaningful for economic, social and environmental benefits. Several technologies have been developed as efficient pretreatments on refractory lignocellulosic materials for enhanced biogasification during the last decades (Hendriks and Zeeman, 2009; Zheng et al., 2014). Figure 1.1 described the research route of this study.

1.3. Technologies applicable for lignocellulosic biomass pretreatment

Many methods including mechanical, chemical and biological pretreatment have been investigated for the hydrolysis of refractory lignocellulosic substances and then biogas production (Hendriks and Zeeman, 2009; Ren et al., 2009; Zheng et al., 2014). Some of these pretreatment methods do exhibit high efficiency in the hydrolysis of lignocellulosic biomass. Still, the following disadvantages hinder their practical application to some extent: (1) Solely mechanical pretreatment is not effective and thus always applied in combination with other methods like chemical or biological pretreatment; (2) Biological pretreatment process is complex and difficult to maintain its efficiency; (3) Chemicals addition in chemical pretreatment usually causes secondary pollution demanding additional wastewater treatment.

1.4. Hydrothermal treatment (HTT)

Among above-mentioned methods, hydrothermal treatment (HTT) has been received considerable attention in recent years. For example, in 1990s Bobleter (1994) got enhanced susceptibility of lignocellulosic material to enzymatic hydrolysis by using HTT pretreatment. As for HTT, liquid water under high temperature and pressure was firstly applied, thus this technology was also called liquid hot water pretreatment, hot compressed water, autohydrolysis, hydrothermolysis process, thermal hydrolysis, pressure-cooking in water, aqueous pretreatments (López González et al., 2014; Sun et al., 2010; Suryawati et al., 2009; Ferreira et al., 2014; Weil et al., 1998a; Overend and Chornet, 1987). Compared with other pretreatments, the major advantages of HTT are non-chemicals addition, handleability, and effective degradation of lignocellulose at low cost (Garrote et al., 1999; Merali et al., 2013; Rogalinski et al., 2008). During HTT process, the lignocellulosic biomass is exposed to high temperature and high pressure, achieving enhanced degradation of hemicellulose and lignin in addition to increased cellulose hydrolysis.

As can be seen from previous studies, most of the related HTT works were performed as pretreatment of lignocellulosic biomass for ethanol production (Sun and Cheng, 2002; Zhao et al., 2014). Only in recent years the number of studies of using HTT for biogas production increased significantly, showing a rising interest in this conversion technology to enhance biogasification. Figure 1.2 shows the schematic diagram of hydrothermal pretreatment of lignocellulosic biomass for anaerobic biogasification. On the other hand, although some studies have addressed and compared the difference generally in various pretreatments of lignocellulosic biomass for biogas production (Hendriks and Zeeman, 2009; Taherzadeh and Karimi, 2008; Zheng et al., 2014), no special report could be found on recent HTT pretreatment studies in detail for biogas production from lignocellulosic biomass, especially the HTT operation conditions which are crucial for bio-methane production from biomass. Therefore, this part is to present recent research progress on hydrothermal processing of lignocellulosic biomass for biogas production. And the following two aspects will be emphasized and discussed: (1) Effects of HTT on the degradation of cellulose, hemicellulose and lignin; (2) Effects of HTT operation conditions on methane production from lignocellulosic biomass.

1.4.1. Effect of HTT on the conversion of lignocellulosic biomass

Lignocellulosic biomass consist three main components, i.e. cellulose, hemicellulose and lignin with their contents up to 50-90%. Table 1.1 lists the contents of cellulose, hemicellulose, and lignin in various lignocellulosic biomass. For anaerobic digestion, the cellulose crystallinity, accessible surface to enzymes and the structure and distribution of lignin will affect the biodegradability of lignocellulosic biomass (Wyman, 1996; Hendriks and Zeeman, 2009; Fernandes et al., 2009). Therefore, it is necessary to understand the effects of HTT on cellulose, hemicelluloses and lignin, and then the hydrothermal changes of lignocelluloses.

(1) Cellulose

Cellulose is the main component of lignocellulosic biomass, accounting for around 11%-53% (dry matter) (Table 1.1). It is a linear polymer which consists of glucose units, linked by β-(1→4)-glycosidic bonds (Delmer and Amor, 1995; Morohoshi, 1991). Previous studies have shown that liquefaction behavior of cellulose occurs at temperature of 200-350℃. Cellulose begins to decompose at 200℃, and the reaction rate is faster at higher temperature (＞240℃) than that at lower temperature (＜240℃) (Minowa et al., 1998; Minowa et al., 1997). This reaction can be completed around 280℃, and the formation of oil, gas and char starts from 240℃ (Minowa et al., 1998; Minowa et al., 1997). Moreover, some similar results have also been obtained from other researchers. Sakaki et al. (2002) found that cellulose began to decomposed into a water-soluble fraction when temperature >230℃ and the reaction can be completed nearly at 295℃. Jin et al. (2004) pointed out that cellulose was hydrolyzed into glucose in 120 s at 300℃ under 8.9 MPa, and the latter was further decomposed in 30 s. Rogalinski et al. (2008) studied hydrolysis kinetics of different biopolymers, and claimed that hydrolysis of cellulose was enhanced with increasing temperature from 240 to 310℃ at 25 MPa.

Gao et al. (2012) examined the products from hydrothermal treatment of cellulose at a temperature range of 200-400℃ and residence times of 5 min-2 h, with aldehydes, phenols, ketones, acid groups and sugars being detected in the aqueous phase. The D-glucose originating from cellulose can be thermally degraded directly during hydrothermal pretreatment: firstly being dehydrated to 5-hydroxymethyl-2-furaldehyde (HMF) which then is further degraded to formic acid and levulinic acid (Rasmussen et al., 2014; Yang et al., 2012). Efficient pre-treatments should avoid loss of carbohydrates, meanwhile minimize the accumulation of rate limiting compounds (e.g. furfurals, HMF etc.) which are associated with slower kinetics in methane production (Benjamin et al., 1984).

For instance, the concentration of furfural has been quantified in the HTT of different biomass. According to the calculation of López González et al. (2014): when switchgrass was pretreated at 200℃ for 10 min, the furfural concentration was 0.72 g/100 g switchgrass (Suryawati et al., 2009); In other studies, when the temperature varied from 200 to 220℃ for 5 - 15 min, the furfural concentration was reported to be 0.2 - 3.1 g/100 g biomass (Nitsos et al., 2013; Pérez et al., 2007; Suryawati et al., 2009; Yu et al., 2010a). Correspondingly, López González et al. (2014) investigated the effect of HTT treated sugarcane press mud on methane production. They obtained the maximum furfural concentration of 1.21 g/L (0.73 g/100 g press mud) at 200℃ for 5 min. Meanwhile, very low methane yield was detected after pretreated above 200℃, which was probably relating to the inhibited methanogenesis by the formation of refractory compounds at high HTT temperature (López González et al., 2014). Bougrier et al. (2008) also reported that at high temperature, some colored recalcitrant compounds could be produced via Maillard reaction by polymerization between carbohydrates and amino compounds. And these colored recalcitrant compounds were found to be unfavourable for methanogenesis (Rodríguez-Abalde et al., 2011).

(2) Hemicellulose

Hemicellulose represents the second main component of lignocellulosic biomass with a content of about 3%-56% (dry matter) (Table 1.1). Hemicelluloses are polysaccharides and consist of xyloglucans, xylans, mannans and glucomannans, and β-(1→3, 1→4)-glucans (Scheller and Ulvskov, 2010). The specific structure of the hemicelluloses and their abundance vary widely between different species and cell types (Scheller and Ulvskov, 2010). Because of the monomeric substituents (hexoses and pentoses) contained, hemicelluloses may be degraded into HMF, formic acid and levulinic acid. In contrast, furfural is formed exclusively from pentoses, that is, mainly from D-xylose and L-arabinose of hemicellulose (Rasmussen et al., 2014; Yang et al., 2012).

Compared with cellulose which has a rigid and crystalline form, hemicellulose has a lower molecular weight and shorter lateral chains, and is easily hydrolysable. Hemicellulose is reported to be firstly solubilized when temperature > 150℃, and then lignin follows when temperature around 180℃ (Bobleter, 1994; Garrote et al., 1999). During the HTT pretreatment, water under high pressure can act as an acid to penetrate the biomass, accelerating the hydrolysis of cellulose and solubilization of both hemicellulose and lignin (Kim et al., 2009; Pérez et al., 2007; Taherzadeh and Karimi, 2008; Yu et al., 2010b). The hydrothermal process can generate numerous soluble products, and some of them like furans and phenols are known as inhibitors to anaerobic digestion depending on their concentration and bioavailability (Yu et al., 2010b).

Costa et al. (2014) found that soluble sugars were mainly from the hydrolysis of hemicellulose during hydrothermal treatment of sugarcane bagasse at 150-200℃ for 10-30 min. Results showed that hemicellulose remained in the solid fraction when lignocellulosic materials were pretreated at 100℃; at temperatures above 150℃, however, hemicellulose could be hydrolysed and dissolved into the liquid fraction or hydrolysate (Hendriks and Zeeman, 2009; Fernández-Cegrí et al., 2012). The hemicellulose can be solubilised with the production of acetic acid during HTT, and the produced acetic acid can act as a catalyst in this hydrolysis, which further degrades the polymer resulting in the increase in sugar yield (Hu and Ragauskas, 2011; Mosier et al., 2005c).

Bayr et al. (2013) studied the impact of 12 pretreatments (such as hydrothermal, enzymatic, ultrasound and chemical pretreatments alone or in combination) on methane yield of the secondary sludge from pulp and paper mill wastewater treatment. Interestingly, the highest methane yield (141 ml/g-VS, 31% higher than control) was achieved by hydrothermal pretreatment (10 min at 150℃) alone or in combination with enzymatic and/or ultrasound treatment. Meanwhile, their results showed that all pretreatment caused a decrease in cellulose content, and specifically a significant decrease in hemicellulose content. The reason of enhanced methane yield could be interpreted as the result of efficient solubilization (an increase in soluble chemical oxygen demand) of sludge through HTT. The increase in biodegradability and methane yield after thermal pretreatment can be attributed to the fact that thermal treatment causes disrupting of chemical bonds in cell walls and membranes, and then the release of intracellular organic materials benefits the subsequent biological degradation (Appels et al., 2010).

(3) Lignin

The third compound, lignin, which exists in cell wall, is known to be composed of three different phenylpropane units (p-coumaryl, coniferyl and sinapyl alcohol) that are held together by different kind of linkages (Hendriks and Zeeman, 2009). Lignin, one of the most abundant polymers in nature, is a complex and amorphous heteropolymer, which is also non-water soluble (Hendriks and Zeeman, 2009).

Cellulose and hemicellulose are cemented together by lignin which is responsible for supporting plant structural and against microbial attack and oxidative stress (Taherzadeh and Karimi, 2008). Due to the lignocellulosic nature and high content of recalcitrant lignin, the fermentation of lignocellulosic biomass is difficult in practice because of limited enzyme accessibility. Moreover, lignin derivatives with aldehyde groups or apolar substituents are toxic to methanogens, showing inhibition effect on methanogenesis (Op den Camp et al., 1988). From the results of Benjamin et al. (1984), eugenol with an apolar side chain is more toxic to methanogens than guaiacol which lacks the side chain by using the lignin samples extracted from kraft condensate.

In general, hydrothermal treatments at temperature of 150℃ or above firstly lead to partial solubilization of the hemicellulose of biomass, together with which lignin starts to dissolve when temperature increases to around 180℃ (Bobleter, 1994). The solubility of lignin in acid, neutral or alkaline environments depends on its precursors including p-coumaryl, coniferyl, sinapyl alcohol or combinations of them (Grabber, 2005). After hydrothermal pretreatment of lignocelluloses, the dissolved portion of lignin may exert inhibition effect on cellulase, xylanase, and glucosidase. More specifically, the activity of various cellulases can be inhibited differently by lignin, while xylanases and glucosidase are less affected (Berlin et al., 2006).

According to recent researches, regardless of its possible solubilization, the change in lignin content is probably related to solidification and re-deposition on the biomass surface due to cooling after severe pretreatment conditions (Liu and Wyman, 2005; Negro et al., 2003). Thus, no lignin removal but just re-allocation of lignin is possible during the pretreatment at high temperatures (Kristensen et al., 2008). This phenomenon has been observed during the hydrothermal pretreatments of agricultural residues such as switchgrass and paper tube residuals (Kumar et al., 2011; Teghammar et al., 2010).

In addition, the presence of lignin has been reported to have negative effect on the yields of sugars with furans produced from raw corncob. The delignified corncob, however, can have significantly improved sugars yields, indicating lignin plays an important role in the biomass conversion system via sugar platforms (Daorattanachai et al., 2013).

1.4.2. Operating conditions of HTT for subsequent biogas production

A significant drawback of hydrothermal method is the formation of phenolic compounds as well as furfural and HMF at the applied temperature (Gossett et al., 1982; Hendriks and Zeeman, 2009). These byproducts, generally toxic, can inhibit the growth of Bacteria and

Archaea (Hernandez and Edyvean, 2008). The HTT outcome largely depends on retention time, temperature, particle size and moisture content, etc. (Sun and Cheng, 2002). Therefore, in order to reduce the possibility of formation of these inhibitory compounds and obtain the maximum methane yield from lignocellulosic biomass, the temperature, retention time, pressure, solid content, particle size and pH should be optimized during hydrothermal process.

(1) Temperature

Higher temperature will bring about lower pH in water, enabling the release of O-acetyl, acetic and uronic acids from hemicellulose (Pérez et al., 2007). Moreover, the high temperatures also result in the formation of phenolic compounds and furan derivatives. These undesirable products inhibit the activity of Bacteria and Archaea (Negro et al., 2003; Hernandez and Edyvean, 2008). Therefore, the optimum pretreatment temperature should be determined for the subsequent anaerobic digestion.

Budde et al. (2014) carried out a study to evaluate the effect of different pretreatment temperatures (140-220℃ for 5 min) on bio-methane production from cattle waste. They obtained abundant inhibitors or refractory compounds in the hydrolyte resulting in a low methane yield after pretreatment at 220℃, while 58% increase in methane yield was achieved at pretreatment temperature of 180℃. It has been revealed that temperatures above 200℃ caused the formation of phenolic compounds as well as furfural and HMF, inhibiting the growth of anaerobic microorganisms (Hendriks and Zeeman, 2009; Negro et al., 2003; Teghammar et al., 2010). Fernández-Cegrí et al. (2012) investigated the effect of a wide range of pretreatment temperatures (25-200℃) on methane potential of sunflower oil cake, among which 100℃ yielded the highest methane production. However, O-Thong et al. (2012) reported that hydrothermal pretreatment (230℃ for 15 min) was effectively to increase the methane potential of oil palm empty fruit bunches by 29% (from 161 to 208 ml/g-VS).

Ferreira et al. (2014) investigated the methane production from wheat straw by varying HTT temperatures from 170-220℃ for holding 1-15 min, and the optimum condition was determined at 200℃ for 5 min with methane yield increased by 27%. The difference in the above optimum pretreatment temperatures for various biomasses is most probably attributable to the different chemical compositions and structural characteristics of the lignocellulosic biomass. According to the recent studies on hydrothermal treatment of lignocellulosic biomass for biogas production, the final temperature is better to be controlled between 100 and 230℃.

(2) Retention time

Table 1.2 lists the HTT operation conditions used in recent studies for methane production from lignocellulosic biomass, and Table 1.3 presents the maximum methane yields of different lignocellulosic biomass through batch anaerobic digestion tests after being HTT pretreated. As it can be seen from Tables 2.2 and 2.3, in general, the optimum HTT time of various lignocellulosic biomass for methane production is 10~30 min. Compared with other research works, Fernández-Cegrí et al. (2012) used a relatively longer time (4 h) to pretreat sunflower oil cake at a lower temperature (100℃) to produce methane. From Table 1.3, the pretreatment temperature at 100-150℃ for a longer time (5 min-4 h) is effective for sugarcane press mud, yard waste, sorghum, olive husks, and sunflower oil cake. As for other lignocellulosic biomass, such as sugar beet pulp, sugarcane bagasse, wheat straw, giant reed, rice straw and fruit/vegetable waste, however, a higher pretreatment temperature (160-220℃) with shorter time (1-30 min) seems to be more beneficial for methane production. In order to achieve an effective subsequent biogasification from the biomass, it is speculated that there is some correlation between pretreatment temperature and retention time during HTT process.

According to Overend and Chornet (1987), the concept of pretreatment severity (R_0) can be used to interpret the relationship between temperature and retention time during the hydrothermal treatment (Eq. 1-1).

$$R_0 = t \cdot \exp\,[(T\text{-}100)/14.75] \qquad (1\text{-}1)$$

where t is the pre-treatment time (min), T is the temperature (℃), 100 is the base temperature (℃), 14.75 is the conventional energy of activation assuming the overall reaction is hydrolytic and the overall conversion is first order. As Eq. 1 denotes that the logarithm of R_0 (log R_0), defined as the severity factor (Overend and Chornet, 1987) is mainly dependent on time and temperature, theoretically with no consideration of other factors and effects.

In order to avoid the production of inhibitory and degradation-resistant compounds from hydrothermal treatment under severe conditions, it is necessary to optimize the severity for higher methane yield. Moniz et al. (2013) compared the liquors obtained from corn straw at different HTT temperatures (150-240℃) with severity factors of 1.63-4.51 which increased with the increased concentrations of HMF, furfural and phenolics. The produced oligosaccharides dramatically decreased when the severity factors > 4.21. López González et al. (2014) used the hydrothermal pretreated sugarcane press mud for methane production. They got higher methane production at low HTT temperature (140-150℃) for long retention time (12.5 min, 20 min) with severity factor varied between 2.26-2.77 or at higher temperature (175℃) for short retention time (2 min) with severity factor of 2.49. And the highest methane yield of treated press mud was obtained after pretreated at 150℃ for 20 min with mild severity factor (log R_0=2.77). However, the most severe condition (at 200℃ for 20 min and 210℃ for 12.5 min with log R_0=4.25-4.35) resulted in low methane yield. Moreover, Ferreira et al. (2013) achieved the maximum methane production (273 ml/g-VS_{added}) from HTT pretreated wheat straw at 220℃ for 1 min (log R_0=3.5), about 20% increase in methane production compared with raw straw.

All the above research works indicate that for more severe pretreatments with higher severity factor, the biodegradability of lignocellulosic materials may be decreased resulting in low biogasification, probably due to the formation of inhibitory compounds during HTT process.

(3) Pressure

Hydrothermal pretreatment refers to using water as liquid or vapor or both, and heat to pretreat biomass. During HTT, water under high pressure can penetrate into the biomass, make biomass more accessible to hydrolytic enzymes, meanwhile hydrate cellulose and remove most of the hemicellulose and a part of lignin (Taherzadeh and Karimi, 2008; Pérez et al., 2007). The water above critical point (374℃ and 22.1 MPa) is called supercritical water, and usually temperature below 374℃ and pressure of 0.1-10 MPa are adopted in HTT pretreatment for biomethane production. An important factor influencing hydrothermal process is the formation of hydronium ions from water and from organic acids, since high temperatures and pressures will be created during the process, which could accelerate the decomposition of biomass into valuable monomers in a shorter reaction time (Ruiz et al., 2013).

In recent HTT studies, pressures < 5 MPa are usually applied to pretreat lignocellulosic biomass for subsequent bio-methane production. The influence of pressures on the pretreatment of lignocellulosic biomass for anaerobic digestion has not been revealed extensively. Chandra et al. (2012) conducted the hydrothermal pretreatment of rice straw at pressure of 1.55 MPa (200℃ for 10 min), and obtained more than 2 times methane yield (132.7 ml/g-VS) of the untreated straw. Teghammar et al. (2010) compared the effect of explosion (at pressure of 1.5-2.0 MPa) with nonexplosive hydrothermal pretreatment of paper tube residuals for biogas production. The explosive pretreatment at 2.0 MPa and 220℃ for 10 min with addition of both 2% NaOH and 2% H_2O_2 gave the best results with methane yield increased by 70–107% (from 238 to 403-493 ml/g-VS). High pressure is regarded to increase the accessible surface area of biomass to vapor and thus decrease the crystallinity of cellulose.

(4) Solid content

Solid content, namely biomass to water ratio, is a key parameter which influences the efficiency of hydrothermal treatment. As for the pretreatment for methane production, HTT at solids content ⩽ 20% is usually adopted. At high biomass to water ratios, the relative interactions between molecules of biomass and those of water become less influential, which can suppress dissolution of biomass components (Akhtar and Amin, 2011). Laser et al. (2002) performed the hydrothermal treatment of sugarcane bagasse in a 25 l reactor at 170-230 ℃ for 1-46 min with solid content varied from 1% to 8%. The optimal conditions for xylan recovery were high temperature (above 220 ℃), short retention time (＜2 min) and low solid content (＜5%). In addition, Allen et al. (2001) obtained 86% of simultaneous saccharification and fermentation (SSF) conversion and 82% of xylan recovery at a solid content of 5%, and no hydrolyzate inhibition on fermentation yield was detected (the fermentation rate, however, was inhibited). From another point of view, Adl et al. (2012) evaluated the energy recovery from HTT pretreated cotton stalk through anaerobic digestion. Their results indicate that HTT pretreatment is feasible and viable at high solid content (>10%), and >175 ml/g-VS of methane could be recovered from pretreated stalk at higher solid contents, which to some extent is consistent with the finding of Bruni et al. (2010). After investigating the effect of HTT (155-180℃for 15 min) on bio-methane potential of biofibers (a mixture of cow and pig manure, maize silage and industrial by-products) under different initial total solid contents (7.4-14.5%), Bruni et al. (2010) obtained the highest increase in methane yield (by 67% compared to untreated biofibers) at 155℃, 12.4% of total solids (TS), and 2.1% of acid w/w with no pre-soaking. They also recommended that pretreatment at higher solids content (14.5% of TS) and higher temperature (180℃) without acid addition was the most promising condition for full-scale applications. In addition, Ziemiński et al. (2014) used HTT (at 160℃) to pretreat sugar beet pulp at high solid content (TS~33%) in a lab-scale thermostatic reactor for subsequent methane production, yielding the highest methane production of 502.5 ml/g-VS).

(5) Particle size

Particle size reduction is considered as a factor which can enhance the accessibility of lignocellulosic biomass to hydrothermal treatment due to higher specific surface area created, thus increase lignocellulose conversion rates for methanogenesis. However, the realization of size reduction needs energy input, therefore it is necessary to optimize the particle size of biomass for hydrothermal pretreatment. In general, the reported maximal particle size follows a descending order among the following pretreatments: Steam explosion > hydrothermal > dilute acid and base pretreatments (Vidal Jr. et al., 2011). In addition, the maximal size is also dependent on feedstock itself, and herbaceous or grassy biomass has smaller maximal size (< 3 mm) than woody biomass (>3 mm) (Vidal Jr. et al., 2011). Khullar et al. (2013) investigated the effect of particle size on enzymatic hydrolysis of pretreated *Miscanthus*. They found that glucose concentration, glucose release rate, glucose yield and total conversion were increased with the decrease in particle size during HTT (at 200℃ for 30 min): The highest glucose concentrations and hydrolysis were achieved from the smallest particle (0.08 mm) *Miscanthus*. Lamsal et al. (2010) suggested that particles size <132 μm was favorable for soluble sugar release from the hydrolysis of three feedstocks (soybean hulls, wheat straw, and de-starched wheat bran).

On the other hand, some research works also reflect that the effect of particle size on sugar yield is not so significant during the HTT pretreatment of lignocellulosic biomass. Ruiz et al. (2011) pointed out that the particle size of wheat straw didn't influence sugar degradation, but had a selective effect on the extraction of total sugars. In addition, no difference in corn stover glucose conversion (%) was found between two particle sizes (53-75 μm and 425-710 μm) during HTT at 190℃ for 15 min, and pH 4.3-6.2. The HTT pretreatment brought about the change in plant cell ultrastructure and the formation of micron-sized pores, making cellulose more readily hydrolysable (Zeng et al., 2007). Excessive fine grinding requires energy in quantity and it is not recommended for hydrothermal pretreatment. Ferreira et al. (2014) investigated the impact of particle size on bio-methane potential of wheat straw, and their results showed that larger particles (cutting 30-50 mm) were better than small ones (milling <1 mm). Moreover, seen from economic viewpoint, it is absolutely negative by milling biomass before HTT pretreatment, which definitely results in the increase of operation cost. For hydrothermal liquefaction, biomass particles are recommended to be 4-10 mm, which is suitable to overcome heat and mass transfer limitations at a reasonable grinding cost (Akhtar and Amin, 2011).

(6) pH and chemicals' addition

In order to avoid the formation of inhibitors during hydrothermal pretreatment and to mainly solubilise hemicellulose thus to make cellulose more accessible to hydrolysis, it's necessary to maximize the solubilisation of hemicellulose into soluble oligosaccharides while minimize the hydronium ions concentration and, more importantly, the degradation of the resultant oligosaccharides and monosaccharides to smaller products (Weil et al., 1998b; Mosier et al., 2005a; Mosier et al., 2005b; Hendriks and Zeeman, 2009; Ruiz et al., 2013; Li et al., 2014; Zheng et al., 2014). Mosier et al. (2005a) successfully used pilot plant scale pH-controlled hydrothermal treatment for ethanol production from corn fiber at 160℃ and pH ＞4.0, achieving 50% dissolution of the fiber in 20 min. The carbohydrates they obtained were composed of 80% soluble oligosaccharides and 20% monosaccharides. Weil et al. (1998a) developed a continuous pH monitoring system for hydrothermal treatment of corn fiber: when 2.0 M KOH was added into the system to keep pH 5.0-7.0, the enzymatic hydrolysis gave 33-84% conversion of cellulose to glucose in the pretreated fiber compared to 17% in untreated fiber. Li et al. (2014) applied pH pre-corrected liquid hot water to pretreat corn stover, and obtained high hemicellulose recovery and low inhibitors formation. Their results indicated this pretreatment reduced hemicellulose degradation by 35.3-92.3% and decreased furfural formation by 90.5-99.8%. Up to now, however, no research has been conducted on pH controlled HTT for the pretreatment of lignocellulosic biomass and then subsequent anaerobic digestion (Zheng et al., 2014).

When pH decrease after hydrothermal treatment is taken into consideration, maintaining suitable pH in subsequent methane production is necessary. Chandra et al. (2012) found that hydrothermal pretreatment of rice straw followed by addition of sodium hydroxide into the fermentor resulted in an increase of 225.6% in biogas production and 222.0% in methane production compared to untreated rice straw. Similarly, hydrothermal pretreatment of paper tube residuals with 2% sodium hydroxide improved the methane yield by 21% from 222 to 269 ml/g-VS (Teghammar et al., 2010). On the other hand, acid-hydrothermal and alkaline-hydrothermal pretreatment were applied to evaluate the methane potential of palm oil mesocarp fibre, and the maximum methane yield (199 ml/g-substrate) was achieved by acid-hydrothermal pretreatment (1.97 M HCl+34 min at 103℃) (Costa et al., 2013). In addition, methanogenic inhibition occurred after hydrothermal giant reed was pre-treated with H_2SO_4 at 2% w/w, which was associated with high SO_4^{2-} concentration in the hydrolysate (in which furfurals were also detected) (Di Girolamo et al, 2013).

1.4.3. Problems and challenges

The HTT outcome largely depends on retention time, temperature, particle size and moisture content, etc. (Sun and Cheng, 2002). Therefore, in order to reduce the possibility of formation of these inhibitory compounds and obtain the maximum biogas production from lignocellulosic biomass, operating conditions should be optimized during hydrothermal process.

Among all the operation condtions, temperature is considered as the most important parameter in pretreatment of biomass for enhanced methanogenesis. Temperatures above 200℃ caused the formation of phenolic compounds, which could inhibit the growth of anaerobic microorganisms. The final HTT temperature is better to be controlled between 100℃ and 230℃ for an enhanced subsequent biogasification. Temperature and retention time are found to be co-related during hydrothermal treatment. In order to avoid the production of inhibitory and degradation-resistant compounds, long time at low temperature or short time at high temperature (with moderate severity) is favorable for hydrothermal treatment when taking subsequent biogasification into consideration.

In addition, pressure, solid content, particle size and pH also influence the hydrothermal pretreatment of lignocellulosic biomass. A higher pressure could accelerate the decomposition of biomass into monomers in shorter retention time, resulting in an increased accessible surface area of biomass to vapor and thus a decrease in the crystallinity of cellulose. Regarding to solid content, higher TS content is beneficial for higher methane yields and practical viability of the pretreatment operation for anaerobic digestion. As for particle size, excessive fine grinding requires energy in quantity and it is not recommended for hydrothermal pretreatment. Finally, in order to avoid the formation of inhibitors during hydrothermal pretreatment and mainly solubilise the hemicellulose thus to make the cellulose more accessible to hydrolysis, maintaining a relatively neutral pH level during HTT is necessary and important.

As reviewed, most of the recent researches focused on the effect of hydrothermal pretreatment conditions on biomethane production and the optimization of HTT conditions for biomethane production. Due to the complexity of structure and the heterogeneity of various biomasses, the following aspects should be considered in future researches in order to realize the cost-effective utilization of HTT pretreatment for biomass to energy production. Firstly, the optimization of operation conditions of HTT pretreatment is still necessary for various lignocellulosic materials. More importantly, how the operation pressure functions and whether pressure-controlled HTT could reduce energy consumption or not is unclear. In addition, it's also important to identify the HTT products and their biodegradability under different operation conditions, and to explore the response of microbial community in subsequent biogasification in addition to the inhibition mechanisms of the intermediates produced during HTT (i.e. furfural, HMF, and phenolic compounds) to anaerobic microorganisms. Finally, a full analysis of environmental, economic and energy efficiencies of HTT pretreatment should be carried out, which is a prerequisite for its practical application in the conversion of biomass to bioenergy.

1.5. Objectives and originality of this study

The objectives of this study are listed as follows: (1) To investigate the influence of temperature and solid content on the soluble products after HTT pretreatment of rice straw and examine the feasibility of biohydrogen production from the pretreated rice straw. Meanwhile, the optimum pretreatment conditions of rice straw for hydrogen production were discussed; (2) To explore the effects of peak temperature and holding time on solubilization of rice straw and subsequent H_2 production and to investigate the relationship of H_2 yield with the soluble products from rice straw during HTT pretreatment; (3) The performance from HTT with subsequent H_2/CH_4 productions were analyzed. More importantly, the performance of single-stage and two-stage fermentation was also compared.

The originality of this study lied in the following three points. Firstly, up to now, little information could be found on the effect of HTT pretreatment on rice straw for bio-hydrogen production. Renewable energy, especially biohydrogen, is a noncarbon based energy resource and regarded as a high efficient fuel to replace fossil fuels. This study improved biohydrogen production of rice straw via HTT pretreatment. The influence of temperature and solid content on the composition of the hydrolysate after HTT pretreatment of rice straw was investigated. Secondly, no result about HTT effect on rice straw could be inferred from the literature for 0 min of holding at the designed HTT temperature. That is, the necessity of a longer holding time for HTT treatment should also be confirmed, which will greatly influence the reactor design and energy input. The effects of peak temperature and holding time on solubilization of rice straw and subsequent H_2 production were explored. And the relationship of H_2 yield with the soluble products from rice straw during HTT pretreatment was also discussed. Finally, although HTT has been proved to be a favorable pretreatment for hydrogen production, a large proportion of the total SCOD still hasn't been utilized for other purposes like methane fermentation. The feasibility of effluent from hydrogen fermentation for subsequent methane productions was also analyzed in the present study.

1.6. Structure of the thesis

The main contents of this study were divided into following parts: (1) In Chapter 2, the preliminary experiments were conducted for investigating the effect of different HTT conditions, including temperature (190-290℃), holding time (1.5 min) and solid content (4-20%) on rice straw pretreatment. In addition, batch experiments of mesophilic fermentation were also carried out to verify the enhancement on biogasification when HTT pretreated rice straw was used as feedstock; (2) In Chapter 3, the followed-up experiments were conducted on the effects of HTT pretreatment at two peak temperatures (150℃ and 210℃) with holding time of 0-30 min on the solubiliztion of rice straw and subsequent biohydrogen production. And the relationship of H_2 yield with the soluble products from rice straw during HTT pretreatment was also discussed; (3) In Chapter 4, the effluent of hydrogen fermentation of HTT pretreated rice straw substrate was used for methane fermentation. The feasibility of this kind of two-stage anaerobic digestion was discussed. In addition, the conventional single-stage along with the single-stage and two-stage fermentation used in this study was also evaluated. The structure of the thesis is shown in Figure 1.3.

Table 1.1 The contents of cellulose, hemicellulose, and lignin in various lignocellulosic biomass.

Lignocellulosic biomass	Cellulose (% TS)	Hemicellulose (% TS)	Lignin (% TS)	References
Wheat straw	46.3-49.6	27.1-29.7	6.2-6.6	Triolo et al., 2011
Rice straw	25.7-30.2	11.9-19.5	17.8-29.6	Sakdaronnarong et al., 2014; Monlau et al., 2012
Maize stalks	41.0	26.0	11.5	Darwish et al., 2012
Sugarcane bagasse	33.3-35.5	14.9-15.9	27.2-31.0	Sakdaronnarong et al., 2014
Sorghum	12.5-29.4	18.1-26.2	17.6-24.1	Monlau et al., 2012
Sunflower stalks	28.1-34.3	11.9-15.9	27.5-35.4	Monlau et al., 2012
Rape straw	37.6	31.4	21.3	Greenhalf et al, 2012
Switch grass	36.0	31.6	6.1	Greenhalf et al, 2012
Cotton stalk	11.4-28.9	30.5-56.0	20.0-32.0	Adl et al., 2012
Corn stover	36.6-37.4	30.7-31.9	17.6-18.0	Saha et al., 2013
Sugar beet pulp	27.4-32.4	24.98-28.62	2.5-5.7	Ziemiński et al., 2014
Cattle waste	15.4-17.9	3.0-9.5	6.1-14.3	Triolo et al., 2011
Grass silage	34.3	29.3	8.6	Xie et al., 2011
Cassava residues	24.49-25.35	17.56-18.12	12.06-12.5	Zhang et al., 2011
Paper tube residue	53	10	23	Teghammar et al., 2010
Populus tomentosa	43.70	18.72	27.71	Wang et al., 2013
Bamboo waste	36.3	12.7	18.7	Shen et al., 2014
Wood chips	39.9-42.5	24.1-26.3	27.6-31	Moniruzzaman and Ono, 2013

TS: Total solid.

Table 1.2 Operation conditions of HTT for subsequent methane production from lignocellulosic biomass.

Lignocellulosic biomass	Temp. (℃)	Retention time (min)	Particle size (mm)	Solid content	Pressure (MPa)	References
Cotton stalk	100	15	＜3	10-16% TS	---	Adl et al., 2012
Rice straw	200	10	＜1	10% TS	1.55	Chandra et al., 2012
Sugarcane bagasse	150-200	10, 20, 30	---	10%m/v (mass/catalyst solution volume)	---	Costa et al., 2014
Giant reed	150,180	10, 20	10	100 gTS with 500 ml volume	---	Di Girolamo et al., 2013
Sunflower oil cake	25-200	240	---	20g/l and 40g/l	---	Fernández-Cegrí et al., 2012
Wheat straw	170-220	1-15	30-50 or ＜1	---	0.5-2.3	Ferreira et al., 2014
Sugarcane press mud	140-210	2-23	---	15% TS	---	López González et al., 2014
Sugar beet pulp	120-220	20	---	100 gTS with 300 ml water	---	Ziemiński et al., 2014
Barley straw	130-190	5-30	＜10	2 kg straw/6 l water	---	Schumacher et al., 2014
Paper tube residuals	190-220	10, 30	1-2	50 g/l	---	Teghammar et al., 2010

TS: Total solid. ---No data in the literature

Table 1.3 Maximum methane yields of different lignocellulosic biomass by batch anaerobic digestion tests after hydrothermal pretreatment.

Substrate	Inoculum	Methane yield (ml/g-VS)	Pretreatment	Anaerobic conditions			Reference
				Temp. (℃)	HRT(d)	Initial pH	
Sugar beet pulp	AD sludge	502.50	160℃, 20 min	37	25	7.2	Ziemiński et al., 2014
Sugarcane bagasse	AD sludge (based on sewage, brewery effluent, glycerol)	197.5 ml/g-substrate	200℃, 10 min	35	30	---	Costa et al., 2014
Palm oil mesocarp fibre	AD sludge (based on wastewater, brewery effluent, glycerol)	199 ml/g-substrate	103℃, 34 min, 1.97 M HCl	35	30	---	Costa et al., 2013
Wheat straw	AD sludge	296	200℃, 5 min+steam explosion	35.1	45	---	Ferreira et al., 2014
Barley straw	Digestates of agricultural waste and municipal sewage sludge	251 ml/g-VS	190℃, 30 min	38	34	---	Schumacher et al., 2014
Sugarcane press mud	Cultivated AD sludge	340.80	150℃, 20 min	37.5	30	7.66	López González et al., 2014
Yard waste	AD sludge	119 ml/g-TS	121℃, 30 min	37	60	---	Zhang et al., 2014
Sweet sorghum	AD sludge	288 ml/g-sorghum	121℃, 1 h	35	---	7.0	Antonopoulou et al., 2012
Secondary sludge from pulp and paper mill wastewater treatment	AD sludge	141	150 ℃, 10 min	55	30	---	Bayr et al., 2013

Wheat straw	AD sludge	273	220℃, 1 min	35	35	---	Ferreira et al., 2013
Giant reed	AD sludge (based on animal manure and industrial food waste)	337	180℃, 10 min	53	39	---	Di Girolamo et al., 2013.
Olive husks (mixed with olive mill wastewater and dairy wastewater)	AD sludge	86.4-93.6	134℃, 20 min	37	30	7.5	Gianico et al., 2013
Rice straw	AD sludge	132.7	200℃, 10 min	37	40	---	Chandra et al.,2012
Sunflower oil cake (Solid fraction)	AD sludge	105 ml/g COD_{added}	100℃, 4 h	35	7	---	Fernández-Cegrí et al., 2012
Sunflower oil cake (liquid fraction)	AD sludge	310 ml/g COD_{added}	100℃, 4 h	35	8	---	Fernández-Cegrí et al., 2012
Fruit/vegetable waste	AD sludge	326.0	170℃, 1 h (0.5 h heating +0.5 h holding)	37	15	---	Qiao et al., 2011
Cassava residues	AD thermophilic sludge	248	157.84℃, 20.15 min, 2.99% (w/w H_2SO_4)	55	14	7.2	Zhang et al., 2011
Wheat straw	Digested manure	396	80℃, 6 min+180℃, 15 min+190℃, 3 min	55	60	---	Kaparaju et al., 2009.

AD: Anaerobic digested. TS: Total solid. VS: Volatile solids. ---No data in the literature

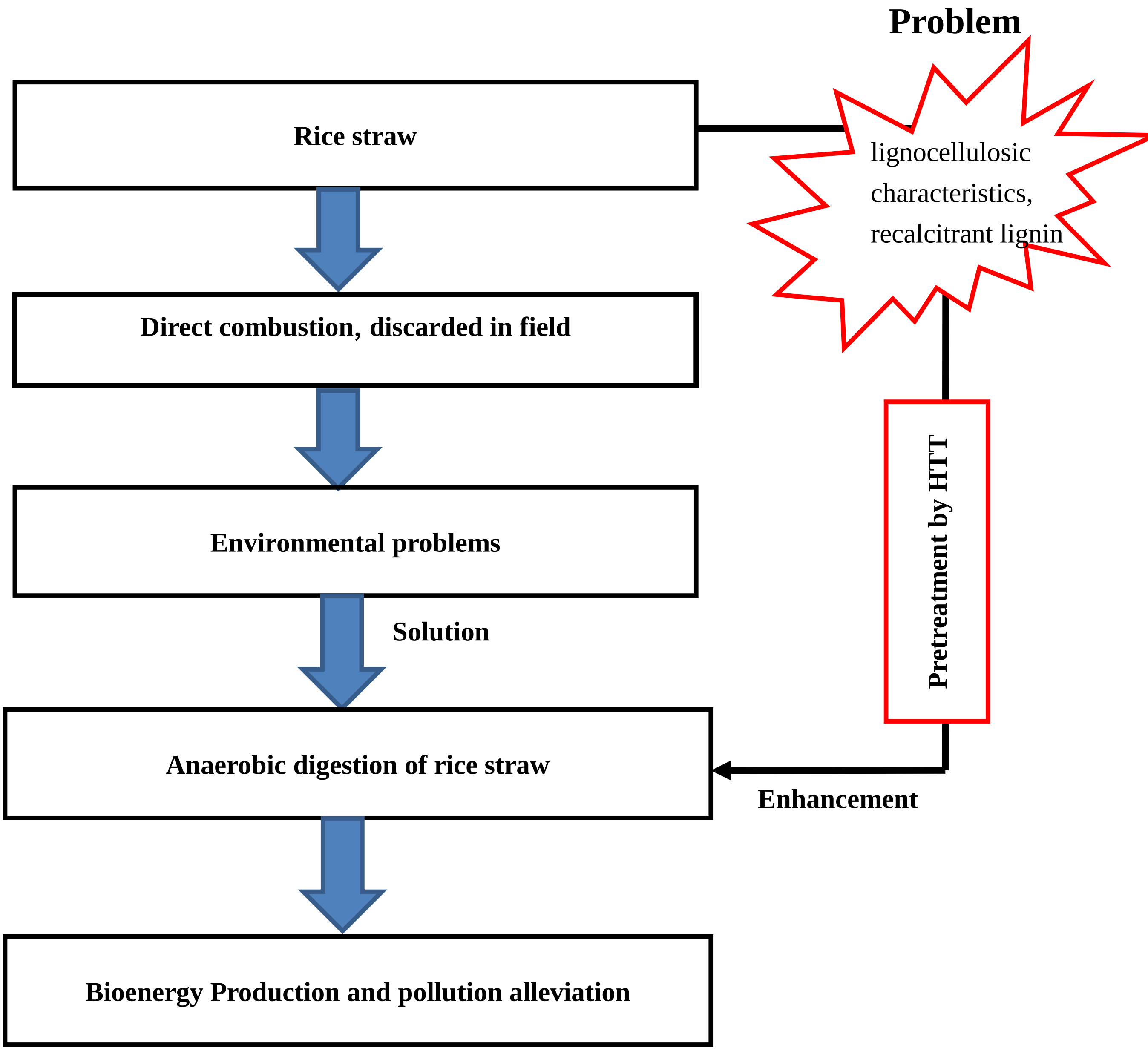

Figure 1.1 Research route of this study

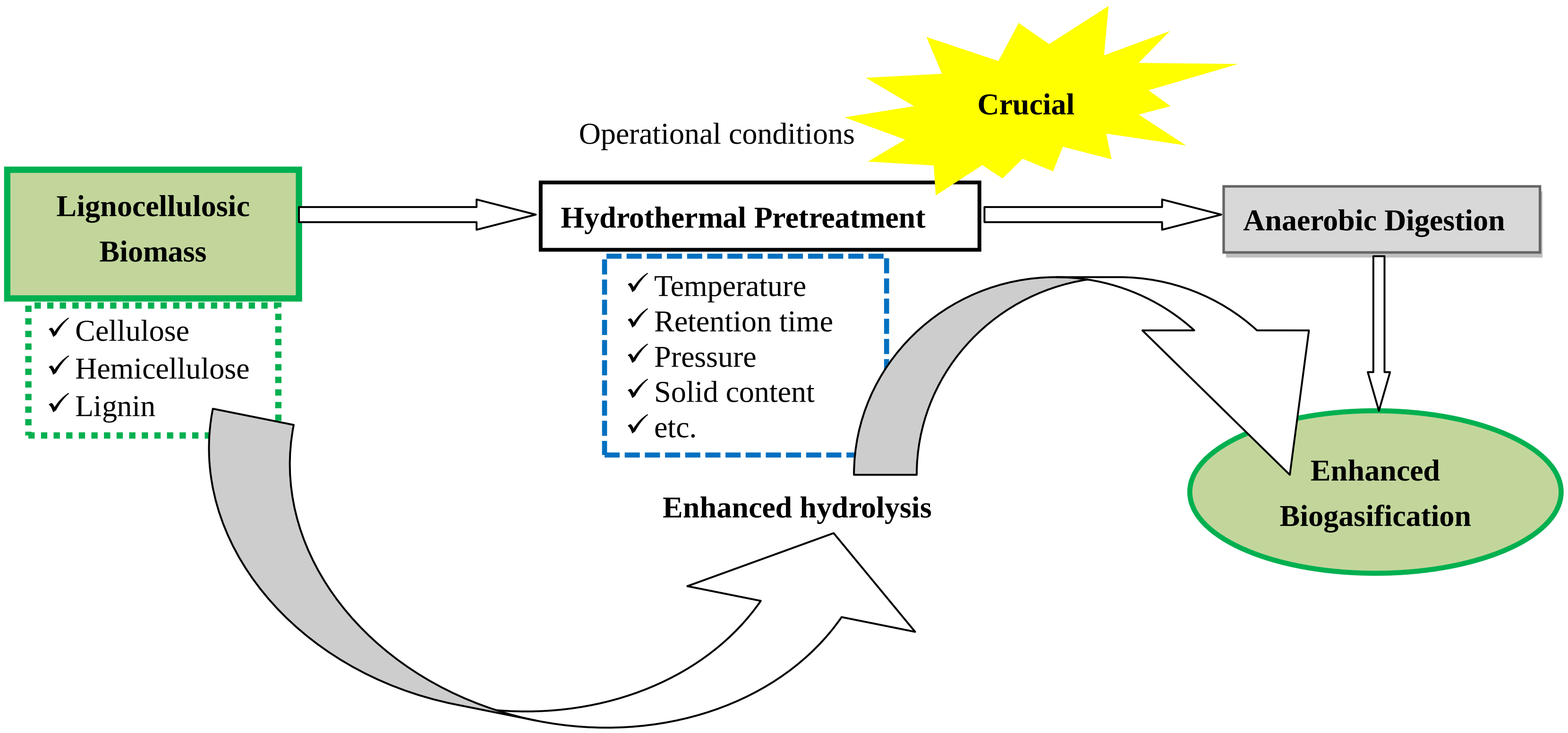

Figure 1.2 Schematic diagram of hydrothermal pretreatment of lignocellulosic biomass for anaerobic biogasification

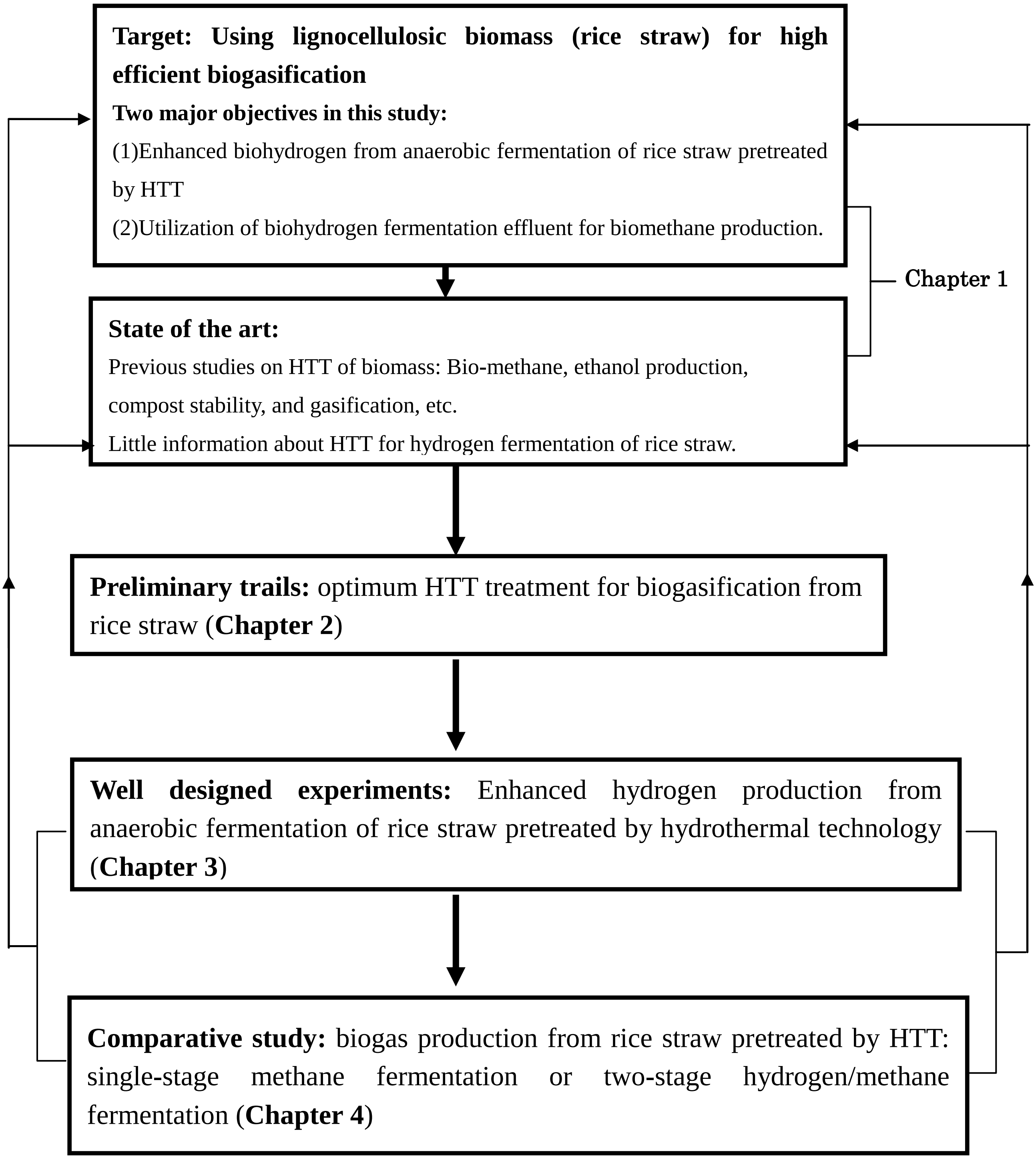

Figure 1.3 Structure of the thesis

2. Preliminary trails for optimum HTT treatment for biogasification from rice straw

2.1. Introduction

Although there are extensive studies on hydrothermal pretreatments of different biomass, most of previous researches have focused on the pretreatment for gasification, bio-methane or ethanol production and compost stability (Chandra et al., 2012; Nakhshiniev et al., 2014; Peterson et al., 2008; Petersen et al., 2009). Few study on HTT pretreatment of rice straw for bio-hydrogen production is available up to now. Due to containing a large amount of cellulose and hemicellulose, rice straw can be used for biohydrogen production by using anaerobic fermentation, a prospective alternative for the sustainable utilization of agricultural waste (Guo et al., 2010). On the other hand, at present, related studies on hydrothermal pretreatment of rice straw applied longer retention time than 5 min for methane production and resulted in higher energy consumption (Chandra et al., 2012; Rodríguez et al., 2009). In this study, in consideration of energy recovery, hydrothermal treatment (HTT) was conducted at short holding time for effective biohydrogen production.

The objective of the study is to investigate the feasibility of HTT pretreatment of rice straw for biohydrogen production. The influence of different operating factors (temperature and solid content) on the composition of the hydrolysate after HTT pretreatment of rice straw was investigated. The biohydrogen production from the pretreated rice straw substrate was examined. The influence of HTT pretreatment on biohydrogen production was alsp discussed.

2.2. Methods

2.2.1. Rice straw and anaerobic sludge

Rice straw was collected from a farm field near to Tsukuba (Ibaraki, Japan), then was cut into particles manually and air-dried. The air-dried rice straw particles were milled to powders and screened by 30-mesh (0.6 mm) and stored at room temperature in dark before hydrogen fermentation experiment. Rice straw was used in this study with TS of 90.1% and volatile solid (VS, of TS) of 79.4%. The anaerobic sludge was obtained from Shimodate Wastewater Treatment Center (Ibaraki, Japan) with TS of 1.1%, and VS/TS of 77.2%.

2.2.2. HTT procedure

The HTT experiments were conducted in a 200 ml enclosed stainless steel reactor with an outer jacket containing electrical wires (Figure 2.1). The reactor was filled with 100 ml distilled water and different weights of rice straw at each time. The TS content of each mixture was adjusted to 4%, 10%, 14%, and 20% respectively. Six different peak temperatures (i.e. 190℃, 210℃, 230℃, 250℃, 270℃, and 290℃) were carried out at each TS level and all the holding time was fixed at 1.5 min. Figure 2.2 describes the schematic diagram of HTT heating process. The pressure was read directly from the installed pressure meter, which was around 1.0, 1.8, 2.5, 4.0, 5.2, and 7.2 MPa, respectively at operation temperature of 190℃, 210℃, 230℃, 250℃, 270℃, and 290℃. After HTT process, the sample was cooled to room temperature, and then the mixture was separated into solid and liquid fractions by vacuum filtration.

2.2.3. Hydrogen fermentation experiment

The pretreated rice straw substrate (a mixture of treated rice straw and soluble substances hydrolysate) under different conditions R190 (210℃, 14% TS, 1.5 min), R210 (210℃, 14% TS, 1.5 min), R230 (230℃, 4% TS, 1.5 min), R290 (290℃, 20% TS, 1.5 min) were inoculated with 150 ml of seed sludge with inoculation ratio ranged between 17-20% based on TS values of the inoculum and the initial fermentation substrate. All these samples were put into 250 ml glass bottle and 2 M sodium hydroxide or hydrochloric acid was used to adjust the initial pH of each reactor to be around 7.0. Their final volume was made up to 200 ml by using deionized water before fermentation. All the fermentation reactors were sealed with silica gel stoppers, and placed in a thermostat controlled at 35±1℃ after their headspace being flushed with nitrogen gas for 5 min. Each stopper was connected with a 50 ml graduated plastic syringe for biogas collection and quantification.

2.2.4. Analytical methods

TS and VS of rice straw before and after HTT pretreatment and TS and VS of seeding anaerobic sludge were determined in accordance with the Standard Methods (APHA, 2005). After HTT process, the resultant substrate was separated into solid and liquid fractions. The liquid fraction was centrifuged at 10,000 rpm for 10 min and the supernatant was used for the determination of soluble chemical oxygen demand (SCOD) and carbohydrate. SCOD was measured in accordance with the Standard Methods (APHA, 2005). Carbohydrate was determined using the phenol-sulfuric method with glucose as standard (Herbert et al., 1971). Morphology characteristics of the rice straw particles before and after HTT pretreatment were observed using a scanning electron microscope (SEM, JSM6330F, Japan). During fermentation, the volume of biogas production was daily read directly from the scale on the syringe after the bottles being shaken manually for 2 min. Biogas composition analysis was carried out by a gas chromatography (GC-8A, SHIMADZU, Japan) with N_2 as carrier gas, equipped with a thermal conductivity detector (80℃) and a Porapak Q column(60℃).

2.3. Results and discussion

2.3.1. Effect of different HTT pretreatment conditions on TS and VS of rice straw

HTT pretreatment is an effective method for degradation of lignocellulosic biomass. Figure 2.3 depicts the changes in TS and VS of rice straw after HTT pretreatment under peak temperature from 190℃ (HTT190) to 290℃ (HTT290) for holding time of 1.5 min. It can be seen that TS and VS contents of rice straw were all reduced at all peak temperatures. TS and VS contents were significantly decreased with increasing temperature. Especially, when the temperature was 290℃, the highest TS and VS reduction was obtained, which was 53% and 22%, respectively.

2.3.2. Soluble products

Hydrothermal treatment is an effective method for the solubilization of biomass (Donoso-Bravo et al., 2011), and the breakdown of macromolecular components is temperature dependent (Wilson and Novak, 2009). Figure 2.4 shows the soluble COD concentration of rice straw substrate after 1.5 min HTT pretreatment at different TS content and peak temperature. From Fig. 2.4 and Fig. 2.5, it can be seen that increasing peak temperature is indeed effective for solubilization of rice straw. However, when the temperature was higher than 250℃, the SCOD concentration declined. Carbonization and gasification of organic matters may contribute to SCOD concentration. Sevilla et al. (2009) demonstrated that organic substances, such as carbohydrates, can be carbonized by means of hydrothermal treatment at temperatures of 220-250℃. In addition, the carbonization of agricultural residues rich in carbohydrates was also observed via thermal treatment, whilst biochar was produced from agricultural residues (Oliveira et al., 2013). On the other hand, the organic component of rice straw is firstly degraded into soluble organic substances, and then a part of these substances are decomposed into gas products further at high temperature. The main gas products consisted of CO_2, CO, CH_4 and H_2 during 250-400℃ hydrothermal treatment of cellulose (Gao et al., 2012). The similar results were observed by utilization of lignocellulosic biomass waste in sub- and super-critical water (Williams and Onwudili, 2006; Castello et al., 2013).

As shown in Fig.2.4, at all test temperatures, the yield of soluble carbohydrates increased with increasing temperature firstly, and then declined from 230℃ to 290℃. The highest yields of carbohydrates were obtained at 210℃ for all different solid content samples. Among the high yields of carbohydrates, the maximum yield is 77 mg/g-VS which was obtained at 210℃ and 14% TS for 1.5 min. The results indicated that peak temperature had significant influence on the carbohydrates production. High temperature could cause non-enzymatic browning of carbohydrates, either via carbohydrates caramelization (pyrolysis) or reaction with N-terminal amines through the Maillard reaction (Martins et al., 2000). Furthermore, soluble carbohydrates produced by the hydrolysis of cellulose and hemicellulose are further decomposed into aldehydes, organic acids and furans in subcritical water at high temperature (Saito et al., 2009; Srokol et al., 2004). Efficient pre-treatments should avoid loss of carbohydrates, meanwhile minimize the accumulation of rate limiting compounds (e.g. furfurals, HMF, etc.) (Benjamin et al., 1984).

2.3.3. Effect of HTT pretreatment on biohydrogen production from rice straw

Figure 2.6 describes the cumulative biohydrogen production from the substrates obtained by HTT pretreatment of rice straw. After 1 day from the start-up, the hydrogen production increased quickly and reached to the peak in R210 digester on day 2. And most of the hydrogen production was achieved in 3 days with corresponding final hydrogen yield of 19 ml/g-VS.

On the other hand, very low biohydrogen production was obtained in R230, R290 and the Control. The results indicated that high temperature (＞230℃) is not favorable for subsequent biohydrogen production. Meanwhile, compared to 190℃, temperature at 210℃ showed higher hydrogen production potential. Bougrier et al. (2008) reported that at high temperature, some colored recalcitrant compounds could be produced via Maillard reaction by polymerization between carbohydrates and amino compounds. And these colored recalcitrant compounds were found to be unfavourable for anaerobic digestion (Rodríguez-Abalde et al., 2011). In addition, several studies reported that furan derivatives were detected after the hydrothermal liquefaction of carbohydrates at 200-400℃ (Asghari et al., 2006; Srokol et al., 2004; Kabyemela et al., 1997). These furan derivatives can negatively affect microbial activity, which have inhibitory influence relative to their concentrations (Monlau et al., 2013; Cao et al., 2010). López González et al. (2014) investigated the effect of HTT treated sugarcane press mud on anaerobic digestion. Very low methane yield was detected after pretreated above 200℃, which was probably relating to the inhibited methanogenesis by the formation of refractory compounds at high HTT temperature. In this study, the high temperature (＞230℃) had negative effect on subsequent biohydrogen production. The results suggest that rice straw treated by HTT at an appropriate temperature is very important for biohydrogen production.

As shown in Fig. 2.4, the maximum yield of carbohydrates was gained at 210℃, and the soluble carbohydrates yields decreased significantly when the temperature exceeded 210℃, which is similar with the variation trend of hydrogen production. Yuan et al. (2011) reported that hydrogen production from wheat straw was well correlated with the degradation of cellulose and hemicelluloses into fermentable carbohydrates. Shi et al. (2010) also claimed that the increase in hydrogen yield was almost consistent with the increase in soluble carbohydrates available from alkali pretreated and raw sweet sorghum stalks. In addition, the findings of Monlau et al. (2012) disclosed a strong positive correlation between soluble carbohydrates and hydrogen yields. Therefore, results from the preliminary experiments suggest that the relationship of soluble carbohydrates and biohydrogen production should be further investigated in the followed-up experiments.

2.4. Summary

In the preliminary experiments, the effect of different HTT conditions, including temperature (190-290℃), short holding time (1.5 min) and solid content (4-20%) on rice straw pretreatment were investigated. In addition, batch experiments of mesophilic anaerobic digestion were also carried out to verify the enhancement on biogasification when HTT pretreated rice straw was used as feedstock. The results showed that: (1) higher temperature was effective for reduction of TS and VS. However, when temperature was above 230℃, soluble chemical oxygen demand(SCOD) did not have significant increase with the increase of temperature; (2) the released soluble products were also detected. Less soluble carbohydrates were obtained at higher temperatures (＞230℃); (3) when considering high hydrogen production and energy recovery, moderate temperature (210℃) and high solid content (20%) in HTT was found to be favorable for subsequent biogasification.

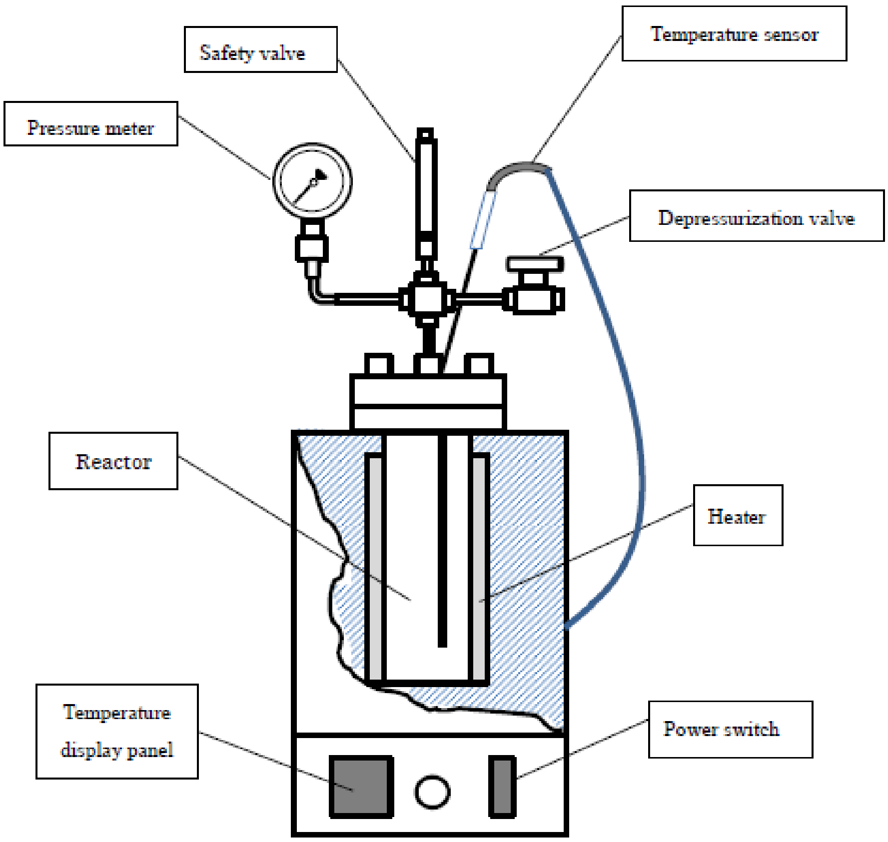

Figure 2.1 Schematic of the hydrothermal reactor in this study

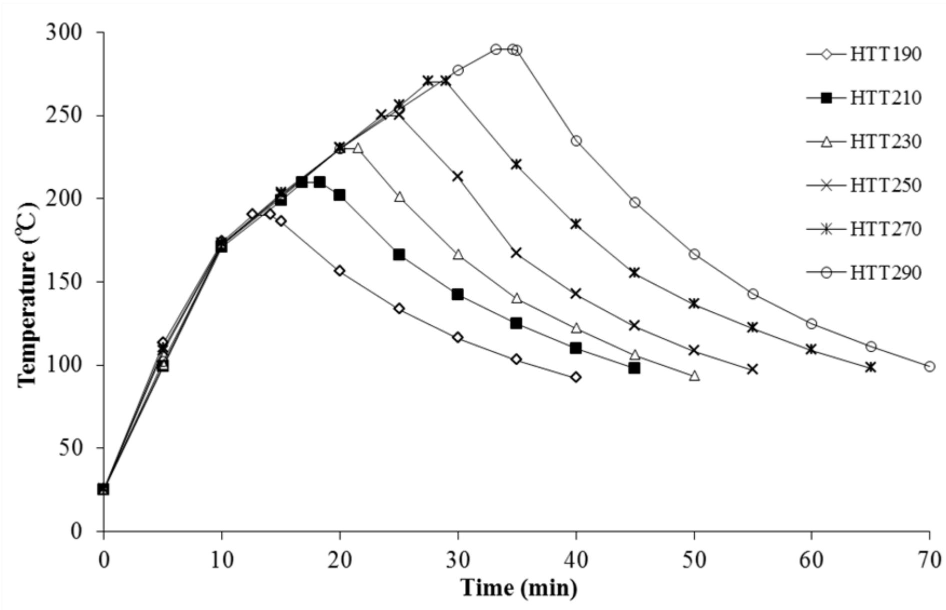

Figure 2.2 Temperature changes in the HTT reactor when peak temperature is controlled at 190℃, 210℃, 230℃, 250℃, 270 and 210℃, respectively.

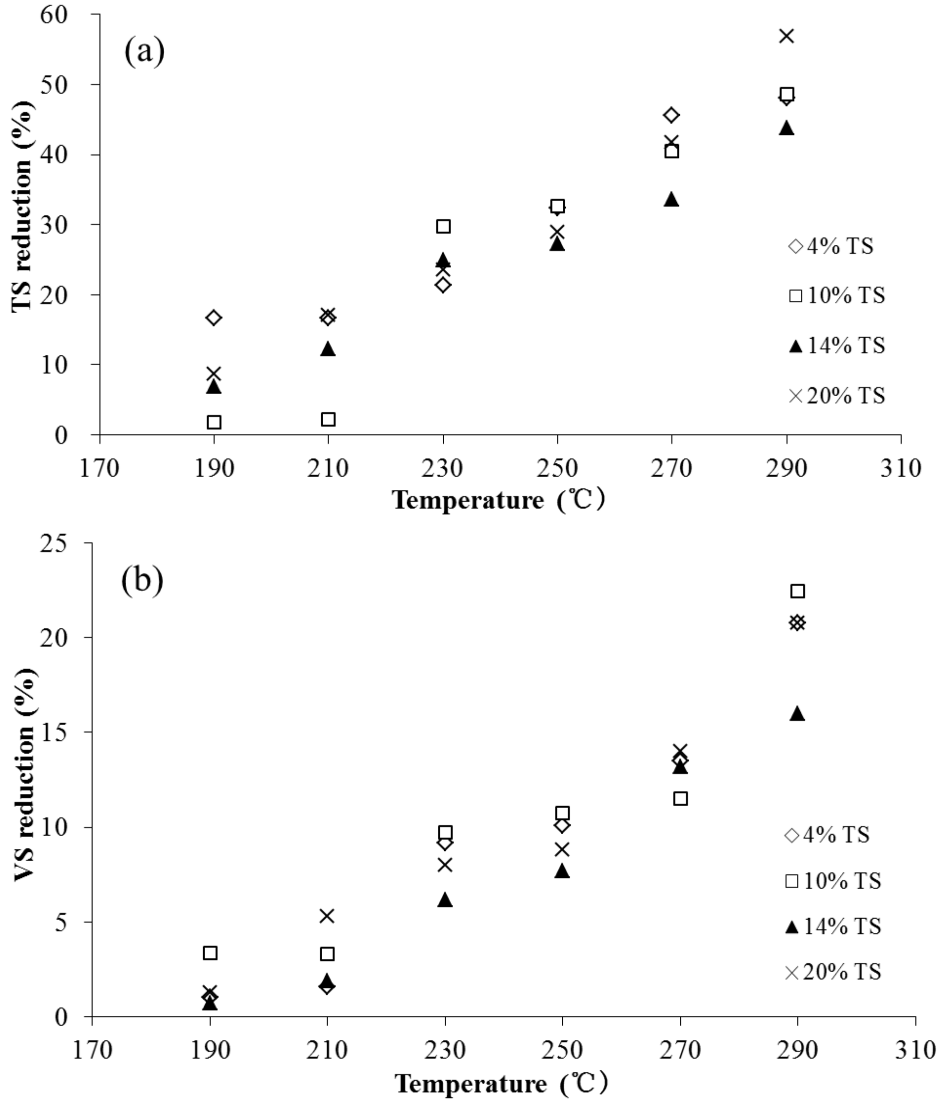

Figure 2.3 Changes in total solids (TS) and volatile solids (VS) of rice straw after hydrothermal pretreatment under peak temperature of 190℃ (HTT190), 210℃ (HTT210), 230℃ (HTT230), 250℃ (HTT250), 270℃ (HTT270) and 290℃ (HTT290) for holding time of 1.5 min, respectively.

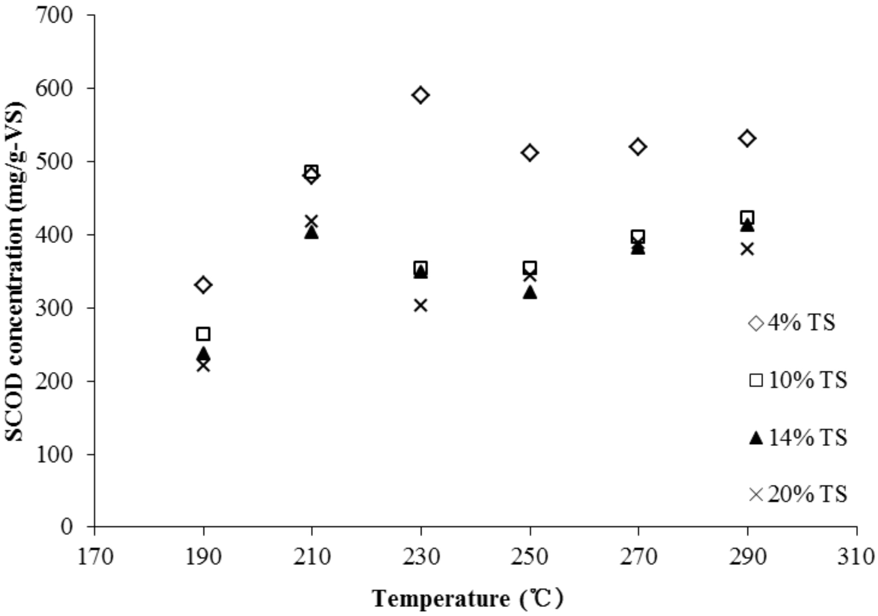

Figure 2.4 Profiles of soluble chemical oxygen demand (SCOD).

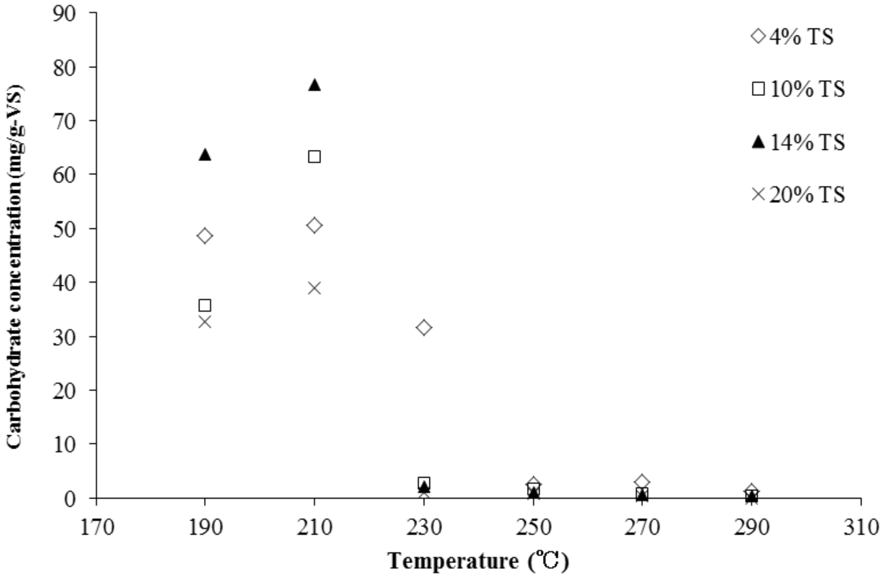

Figure 2.5 Profiles of soluble carbohydrates.

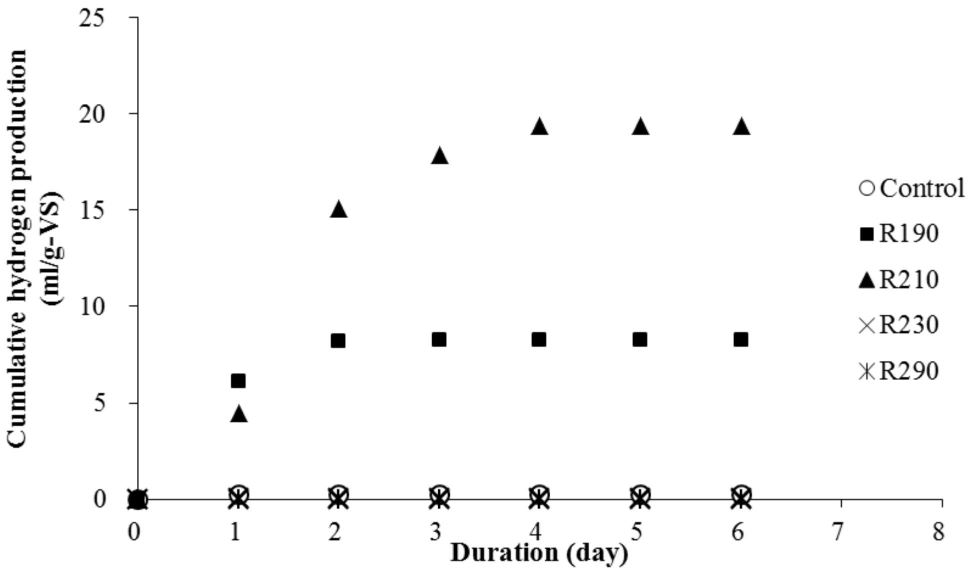

Figure 2.6 Cumulative hydrogen production by using the resultant substrates from HTT pretreatment of rice straw.

3. Enhanced hydrogen production from anaerobic fermentation of rice straw pretreated by hydrothermal technology

3.1. Introduction

Previous studies have been carried out on the HTT treatments of biomass for gasification, bio-methane or ethanol production and compost stability (Chandra et al., 2012; Nakhshiniev et al., 2014; Petersen et al., 2009; Peterson et al., 2008). Among them, Chandra et al. (2012) tried HTT treatment on rice straw biomass at 200℃ for 10 min and achieved enhanced methane production. Nakhshiniev et al. (2014) tested HTT on rice straw at 180℃ for 30 min with improved compost stability. No information about HTT effect on rice straw could be inferred from the literature for 0 min of holding at the designed HTT temperature. That is, the necessity of a longer holding time for HTT treatment should also be confirmed, which will greatly influence the reactor design and energy input.

In this study, HTT, carried out at two designated peak temperatures (150℃ and 210℃, respectively), was holding for 0 min, 10 min, 20 min, and 30 min, respectively. The effects of peak temperature and holding time on solubilization of rice straw and subsequent H_2 production were mainly explored. The relationship of H_2 yield with the soluble products from rice straw during HTT pretreatment was also discussed.

3.2. Methods

3.2.1. Rice straw and seed sludge

Rice straw in this study was collected from a farm field in Tsukuba (Ibaraki, Japan), then air-dried and milled according to Section 2.2.1. The seed sludge was anaerobically digested sludge sampled from the Shimodate Sewage Treatment Center (Ibaraki, Japan). The physical and chemical characteristics of rice straw and anaerobic sludge are shown in Table

3.1.

3.2.2. HTT procedure

HTT pretreatment was conducted in an enclosed stainless steel reactor with working volume of 200 ml. The reactor was loaded with a mixture of 100 ml distilled water and the prepared straw with total solids (TS) content being adjusted to around 20% according to the preliminary experiments. Two different peak temperatures (150℃ and 210℃, labeled as HTT150 and HTT210, respectively with pressure varied between 0.6-1.8 MPa) were applied in HTT experiments at different holding times, i.e. 0 min, 10 min, 20 min, and 30 min, respectively. Fig. 3.1 illustrates the temperature variation in the HTT reactor under each operation condition described in Table 3.2. The temperature in the HTT reactor was averagely elevated at 15.4℃/min and 11.0℃/min during the heating process when HTT peak temperature was controlled at 150℃ and 210℃, respectively. In addition, when conducting holding time of 0 min, the heater was powered off right after the temperature reached to the designated peak temperature.

3.2.3. Hydrogen fermentation

After pretreated under above mentioned conditions (different peak temperature and holding time), the resultant substrate, a mixture of treated rice straw and soluble substances (hydrolysate) produced during the HTT process, was first mixed homogeneously and sampled, and then inoculated with seed sludge. The operation procedure was the same as the previous batch hydrogen fermentation experiment in Section 2.2.3.

3.2.4. Analytical methods

TS and VS of rice straw before and after HTT pretreatment and TS and VS of seeding anaerobic sludge were determined in accordance with the Standard Methods (APHA, 2005).

After HTT process, the resultant substrate was separated into solid and liquid fractions through 1 μm borosilica glass fiber filter (GS-25, Advantec) for the following determinations.

The solid fraction was dried at 105℃ to constant weight, and used for the measurement of lignin, cellulose, and hemicellulose. Lignin was first quantified by gravimetric method after hydrolysis with 72% H_2SO_4 (w/w). The liquor resulted from the hydrolysis process was utilized for the analysis of glucose and reducing sugars which were further used for the estimation of cellulose and hemicellulose contents (Vereris et al., 2007). Glucose and reducing sugars in the liquor were determined with a colorimetric micro-method (Mendel et al., 1954) and a dinitrosalicylic acid (DNS) colorimetric method (Miller, 1959), respectively.

The liquid fraction was centrifuged at 10,000 rpm for 10 min and the supernatant was used for the determination of soluble chemical oxygen demand (SCOD), carbohydrate, and volatile fatty acids (VFAs) after being filtrated through 0.45μm membrane. SCOD and carbohydrates were measured according to the methods in Section 2.2.4. VFAs content was measured using a gas chromatograph (Shimadzu GC-14B) equipped with a flame ionization detector and a Unisole F-200 30/60 column (3.0 mm in diameter and 2.0 m in length). 1 μl of sample was injected with the carrier gas N_2. The injector, detector and column temperatures were kept at 200℃, 200℃ and 160℃, respectively.

Before and after fermentation, the pH values of the substrate were determined using FE20-Kit FiveEasy^{TM} pH meter (METTLER TOLEDO, USA). Morphology characteristics of the rice straw particles before and after HTT pretreatment were observed using a scanning electron microscope (SEM, JSM6330F, Japan). During fermentation, the biogas volume and composition were measured according to the methods mentioned in Section 2.2.4

3.2.5. Statistics

One-way analysis of variance (ANOVA) was applied to determine whether there was significant difference in HTT performance (TS or VS reduction, SCOD or other soluble substances production, etc) among different HTT pretreatment conditions (peak temperature and holding time) or in H_2 yield from the subsequent fermentation of resultant substrate after HTT under the above mentioned conditions. Significance was assumed if the $p < 0.05$.

3.3. Results and discussion

3.3.1. Performance of HTT pretreatment on rice straw

(1) TS and VS reduction

Figure 3.2 depicts the TS and VS reductions of rice straw after HTT pretreatment. The TS and VS contents of rice straw decreased under all tested conditions. More reductions in TS and VS of rice straw were achieved at higher HTT temperature (HTT210), about 10-21% and 3-7%, respectively. Based on ANOVA results, the peak temperature significantly influenced both TS and VS reduction of rice straw during HTT treatment due to $p = 0.0022 < 0.05$.

Clearly seen from Fig. 3.2, the holding time influenced the TS and VS reduction to some extent, especially at higher peak temperature, i.e. 210℃ in this study. After HTT210 pretreatment for 10-30 min, the reductions in TS and VS of rice straw varied between 19-21% and 6-7%, respectively with no significant difference detected among them ($p = 0.4629 > 0.05$). On the other hand, only about 9-10% and 3-4% of TS and VS reductions, respectively were detected in rice straw at the same peak temperature for holding time of 0 min. Results from TS and VS reductions indicated that peak temperature at 210℃ seemed to be more favorable for the hydrolysis of rice straw.

(2)Lignin degradation

In this study attention was also paid to the effect of HTT on the degradation of lignin, one of the major organic components of the rice straw (Table 3.1). Lignin is regarded as a major barrier to the utilization of lignocellulosic biomass in bioconversion process (Zheng et al. 2014). Fig. 3.6 illustrates the change of lignin content induced by the HTT pretreatment. Lignin was about 13-17% of the rice straw before HTT treatment (Table 3.1). After the different tested holding times (0-30 min) at peak temperature of 150℃, no significant change in lignin content was found (p = 0.0732 > 0.05), ranging between 13-14% of the treated rice straw. When HTT was conducted at 210℃, however, the holding time had significant influence on lignin content (p = 3.07E-6 << 0.05). The increase trend of lignin content (from 18% to 29%) in rice straw could be mainly attributed to the following fact: much less TS amount was left in the treated rice straw (Fig. 3.2) while little change in real lignin content. In addition, heterogeneous reaction occurred in the HTT reactor added some difficulty in accurately determining the change in lignin content of rice straw before and after HTT treatment. Being similar with HTT150 treatment, HTT pretreatment at peak temperature of 210℃ had no remarkable contribution to lignin degradation based on the changes in TS and lignin. However, HTT pretreatment did change the morphology of rice straw (Fig. 3.7): the surface of treated straw particles was opened up, which became much rougher, and exhibited obvious porous structures, possibly resulting in much more contacting of water molecules with the carbohydrates inside during HTT process. This observation would contribute not only to the enhanced hydrolysis of hemicellulose and cellulose but also to the increase in microbial accessibility to the carbohydrates contained in rice straw during subsequent biohydrogen fermentation.

(3) Soluble products from rice straw during HTT treatment

In this study the effect of HTT pretreatment on rice straw could be reflected by the changes of SCOD, soluble carbohydrates, and VFAs concentrations in the hydrolysate shown in Fig. 3.3. Obviously, the soluble components in the hydrolysate exhibited a different variance trend with the increase of holding time from 0 to 30 min at the two peak temperatures. Compared to the results from HTT150, more effective solubilization of rice straw was achieved after HTT210 because of their much higher SCOD and VFAs produced (Figs. 4.3a and 4.3b). Interestingly, HTT210 pretreatment with 0 min of holding achieved the highest SCOD and soluble carbohydrates from rice straw, about 420 and 80 mg/g-VS, respectively. No significant difference was observed among the SCOD values from rice straw under HTT210 among the tested holding times (0-30 min) (p = 0.1801 > 0.05). However, when rice straw was treated under HTT150, the highest SCOD and soluble carbohydrates were obtained at holding times of 20 min and 30 min, about 250 and 50 mg/g-VS, respectively, which almost reflected an increase trend with the increase of holding time.

An ideal pretreatment process should achieve high amount of fermentable reducing sugars, avoiding degradation of yielded sugars and less inhibitors for the subsequent fermentation (Hamelinck et al., 2005; Ren et al., 2009). Restated, the soluble carbohydrates was determined to be dramatically decreased under HTT210 from holding time of 0 min to 10-30 min, possibly due to that the produced soluble carbohydrates (such as glucose, sucrose, etc) were further decomposed into smaller molecules at a higher HTT temperature (210℃ in this study). The produced soluble carbohydrates may be originated from the hydrolysis of hemicellulose and then the cellulose of rice straw (Sevilla and Fuertes, 2009) considering the opened-up surface (porous) structure of the treated rice straw (Fig. 3.7) and change in lignin content (Fig. 3.6).

On the other hand, VFAs are important intermediate products during fermentation, which can also be used as substrates for biogas production (Guo et al., 2010). It was found that HTT210 had much more effect on VFAs production from rice straw during HTT treatment (Fig. 3.3b), in which 10-20 min of holding achieved the highest total VFAs of 80-82 mg

COD/g-VS, about ten times of the highest amount of total VFAs under HTT150 conditions (8-9 mg COD/g-VS for 0 min of holding). Among the tested HTT210 conditions, much higher amount of VFAs was detected in the hydrolysate after holding for 10-30 min, especially holding for 10-20 min (Fig. 3.3b). The increase in VFAs production could be contributed by a further decomposition of the produced carbohydrates (Fig. 3.3a). Among the VFAs detected, acetate and propionate were the main VFAs produced from rice straw under all tested HTT conditions, accounting for 83-92% and 96-100% of the total VFAs at peak temperatures of 150℃ and 210℃, respectively. It is worth noting that more propionate rather than acetate was produced under HTT210, while more acetate produced under HTT150. In addition, butyrate was at relatively low levels under all tested conditions, about 0-2 and 0-3 mgCOD/g-VS under HTT150 and HTT210, respectively.

The above results imply that HTT has an effective solubilization effect on lignocellosic materials like rice straw in this study. Different HTT pretreatment conditions achieved different amount of carbohydrates and other by-products from rice straw, which may influence biohydrogen production potentials of rice straw during subsequent hydrogen fermentation. Meanwhile, it also remains unknown whether the simultaneously produced VFAs, especially acetate exhibited inhibition on biohydrogen production or not (Guo et al., 2010; Pampulha and Lourero-Dias, 1989). Thus it is worth conducting the following batch experiments for biohydrogen trials by using the substrate resulted from HTT pretreatment of rice straw (hydrolysate and treated rice straw), further confirming the optimal pretreatment condition.

3.3.2. Batch H_2 fermentation tests by using the resultant substrate from rice straw after HTT pretreatment

Figs.4.4a and 4.4b show the evolution of cumulative H_2 production and H_2 content in the produced biogas from the substrates obtained by HTT pretreatment of rice straw during 6 days' trial. Among these reactors, R210-0 achieved the highest H_2 yield, about 28 ml H_2/g-VS. Namely, HTT pretreatment at peak temperature of 210℃ with holding time of 0 min was the best pretreatment condition for rice straw when taking H_2 production potential into consideration. This observation could be most probably attributed to its highest amount of soluble carbohydrates produced from rice straw (Fig. 3.3a). In addition, after rice straw being HTT pretreated, the biohydrogen production process could be initialed quickly and reached its maximum H_2 yield within 3 days. In contrast, only 0.3 ml/g-VS of H_2 was obtained in the 6 days' test in the control reactor (no HTT pretreatment of rice straw), most probably due to very little soluble substances in the substrate available to H_2 producing bacteria.

Table 3.3 lists the correlation coefficient (r) between the H_2 yield and VS reduction and the content of each soluble product produced from rice straw during the HTT process at the two peak temperature, respectively. Although no obvious relationship was found between the H_2 yield and VS reduction, SCOD and VFAs concentrations, the amount of soluble carbohydrates seemed to have moderately (r = 0.6232) and strongly (r = 0.9987) positive correlation with H_2 yield from the subsequent hydrogen fermentation tests after rice straw being HTT pretreated at peak temperature of 150℃ and 210℃, respectively. Previous studies reported that hydrogen production was well correlated with soluble carbohydrates (Monlau et al., 2012; Shi et al., 2010; Yuan et al., 2011). This work is in agreement with the above-mentioned studies.

In order to confirm whether HTT210 (with holding time of 0 min) did achieve the maximum amount of soluble carbohydrates from rice straw, additional experiments were carried out when HTT peak temperature varied from 150-250℃ with 0 min of holding. As shown in Fig. 3.5, increased amount of soluble carbohydrates was obtained when HTT peak temperature was increased from 150℃ to 210℃, which was decreased remarkably when the peak temperature was further elevated. This observation indicated that cellulose and hemicellulose could be hydrolyzed into soluble carbohydrates under hydrothermal conditions, and the latter can be easily degraded at higher temperature (Figs. 4.3a and Fig. 3.5). The results showed that HTT210 for holding 0 min was the optimal HTT pretreatment condition for subsequent H_2 fermentation in this study.

On the other hand, much less H_2 was produced from other resultant substrates (Fig. 3.4), even though there was a certain amount of SCOD and VFAs (Fig. 3.3) available to the microbes in the reactors. The reason could be interpreted as follows: besides VFAs, some other soluble by-products than carbohydrates were co-existing in the reactor, which amounted to 60-80% of the total SCOD produced from rice straw during HTT pretreatment assuming a COD conversion ratio of 1.191 for carbohydrates (Donoso-Bravo et al., 2011). These co-existing by-products such as furan derivatives might have inhibition effect on biohydrogen production (Asghari et al., 2006; Cao et al., 2010; Kabyemela et al., 1997; Srokol et al., 2004). Thus how to characterize and quantify them, and later utilize them for other purposes like methane production are prerequisite before HTT210 pretreatment being applied in practice.

Table 3.4 lists the maximum H_2 yields from crop straws (rice straw and wheat straw) and their operation conditions. Compared with other effective pretreatments (mainly enzyme addition and acid hydrolysis), HTT pretreatment (at 210℃ with 0 min of holding in this study) possesses the advantages like no chemicals addition, much shorter reaction time and smaller volume of reactor thus lower investment, and relatively lower energy input. The energy balance analysis will be conducted in the near future after combination the economic, social and environmental benefits from HTT pretreatment and subsequent H_2 and methane yields from rice straw.

3.4. Summary

This study investigated the effects of HTT pretreatment at two peak temperatures

(150℃ and 210℃) with holding time of 0-30 min on the solubiliztion of rice straw and subsequent biohydrogen production. The results showed (1) HTT pretreatment at peak temperature of 210℃ could achieve the maximum amount of soluble carbohydrates (80 mg/g-VS) and biohydrogen yield (28 mg/g-VS) when the holding time was 0 min; (2) holding time is crucial for HTT pretreatment combined with subsequent H2 production; (3) Soluble carbohydrates produced from straws during HTT210 was found to have strongly (r=0.9987) positive correlation with the subsequent H_2 yield.

Table 3.1 Physical and chemical characteristics of rice straw and anaerobic digested sludge used in the experiments.

Items	Unit	Rice straw	Sludge
pH	--	N.D.	7.20 ±0.1
Total solid (TS)	%	90.1 ±0.3	1.2 ± 0.0
Volatile solid, VS (of TS)	%	79.4 ±1.1	76.6 ± 0.3
Chemical oxygen demand (COD)	mg/l	N.D.	7206.9 ±374.1
Soluble chemical oxygen demand (SCOD)	mg/l	N.D.	3637.9 ±126.4
Soluble carbohydrates	mg/l	N.D.	22.0 ±1.0
Soluble proteins	mg/l	N.D.	705.3 ±21.2
Lignin	g/100 g-DM	14.6 ±1.9	19.7 ±0.6
Cellulose	g/100 g-DM	28.2 ±0.3	5.1 ±1.7
Hemicellulose	g/100 g-DM	17.5 ±3.1	26.1 ±4.0

DM, dry matter; N.D., no determination.

Table 3.2 Hydrothermal treatment (HTT) and subsequent biohydrogen fermentation conditions applied in this study.

Reactors	HTT pretreatment conditions		Initial solid contents for H_2 fermentation	
	Peak temperature (℃)	Holding time (min)	TS (%)	VS (of TS) (%)
Control	-	-	5.1±0.1	77.2±1.0
R150-0	150	0	5.2±0.1	78.3±0.8
R150-10	150	10	5.4±0.3	78.6±2.5
R150-20	150	20	4.1±0.3	77.2±0.2
R150-30	150	30	4.6±0.2	77.6±2.0
R210-0	210	0	5.0±0.2	75.6±1.6
R210-10	210	10	5.3±0.1	73.6±0.5
R210-20	210	20	5.2±0.3	73.3±0.7
R210-30	210	30	4.7±0.2	74.5±1.2

Control: no pretreatment by HTT. Initial pH of each fermentation reactor was adjusted to be around 7.0.

Table 3.3 Correlation coefficients (*r*) between hydrogen yield (ml/g-VS) and VS reduction and soluble products from rice straw during HTT pretreatment.

Temperature	VS reduction	Soluble COD production	Carbohydrates production	VFAs production
150℃	0.9271	0.9560	0.6232	0.0520
210℃	-0.9842	-0.3767	0.9987	-0.9211

Table 3.4 Maximum H_2 yields from crop straws after different pretreatment by batch anaerobic fermentation tests.

Straw	Inoculum	Hydrogen yield (ml/g-VS)	Pretreatment method	H_2 Fermentation conditions			Reference
				Temperature (℃)	HRT (d)	Initial pH	
Rice straw	AD sludge (no further treatment)	28	HTT at 210℃ for holding 0 min	35	3	7.0	This work
Rice straw	AD sludge (no further treatment)	0.3	No HTT pretreatment	35	3	7.0	This work
Wheat straw	AD sludge after heat-shock treatment at 90℃ for 10 min	19.63	Enzyme addition at 5mg-protein/g-wheat straw	35	6	5.5	Quéméneur et al., 2012
Wheat straw	H_2 producing microflora from cow dung compost	68.1	Acid hydrolysis (2.0% HCl)+ microwave (8 min)	36	5.3	7.0	Fan et al., 2006
Wheat straw	H_2 producing microflora from CSTR	6.4	No pretreatment	36	2	5.2-5.4	Nasirian et al., 2011
Wheat straw	H_2 producing microflora from CSTR	41.9	2% H_2SO_4 + 120℃ for 90 min	36	5	5.2-5.4	Nasirian et al., 2011
Wheat straw	Pure culture (*C. saccharolyticus*)	44.7	130℃, 30 min	70	8	---*	Ivanova et al., 2009

AD, anaerobic digested. CSTR, continuously stirring tank reactor which was producing H_2 by fermenting waste sugar. * No data in the literature.

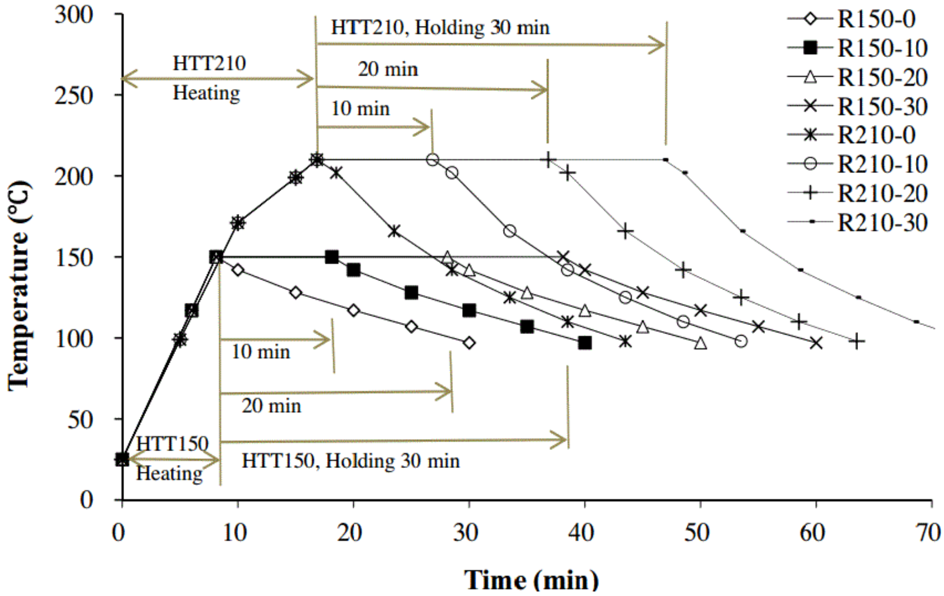

Figure 3.1 Temperature changes in the HTT reactor when peak temperature is controlled at 150℃ and 210℃, respectively.

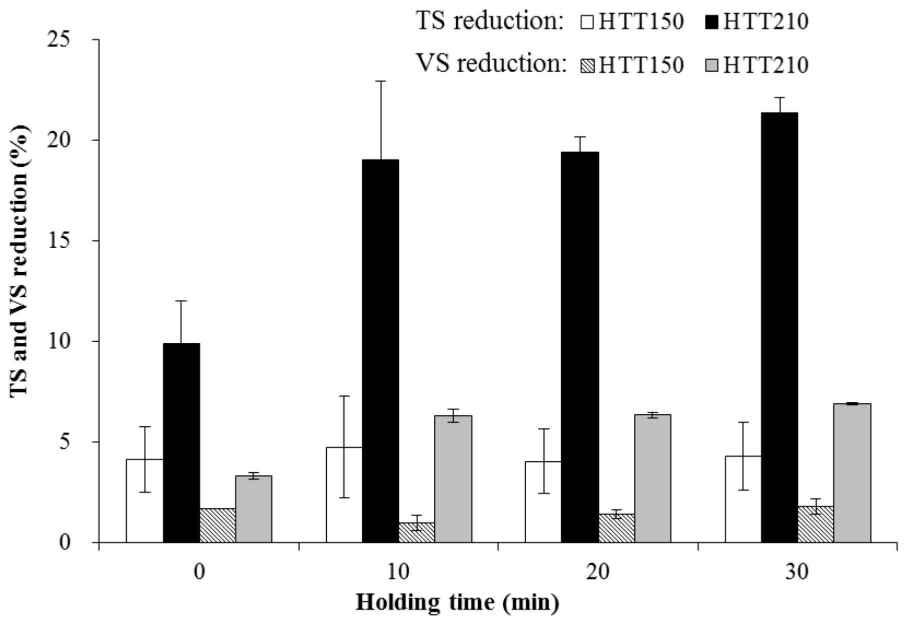

Figure 3.2 Changes in total solids (TS) and volatile solids (VS) of rice straw after hydrothermal pretreatment under peak temperature of 150℃ (HTT150) and 210℃ (HTT210) for holding time of 0-30 min, respectively.

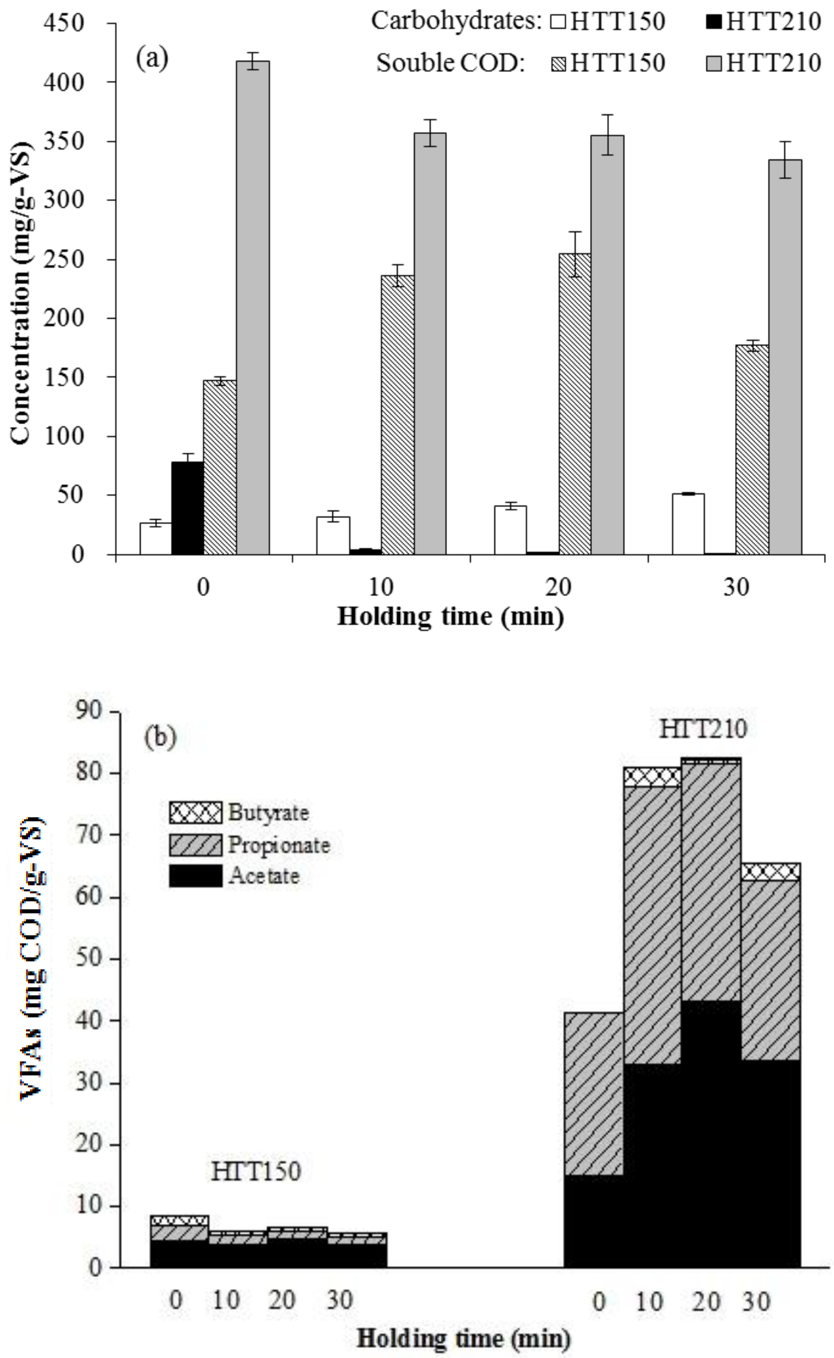

Figure 3.3 Profiles of soluble chemical oxygen demand (SCOD) and soluble carbohydrates (a); Volatile fatty acids (VFAs, b) produced from rice straw during hydrothermal pretreatment under peak temperature of 150℃ (HTT150) and 210℃ (HTT210) for holding time of 0-30 min, respectively.

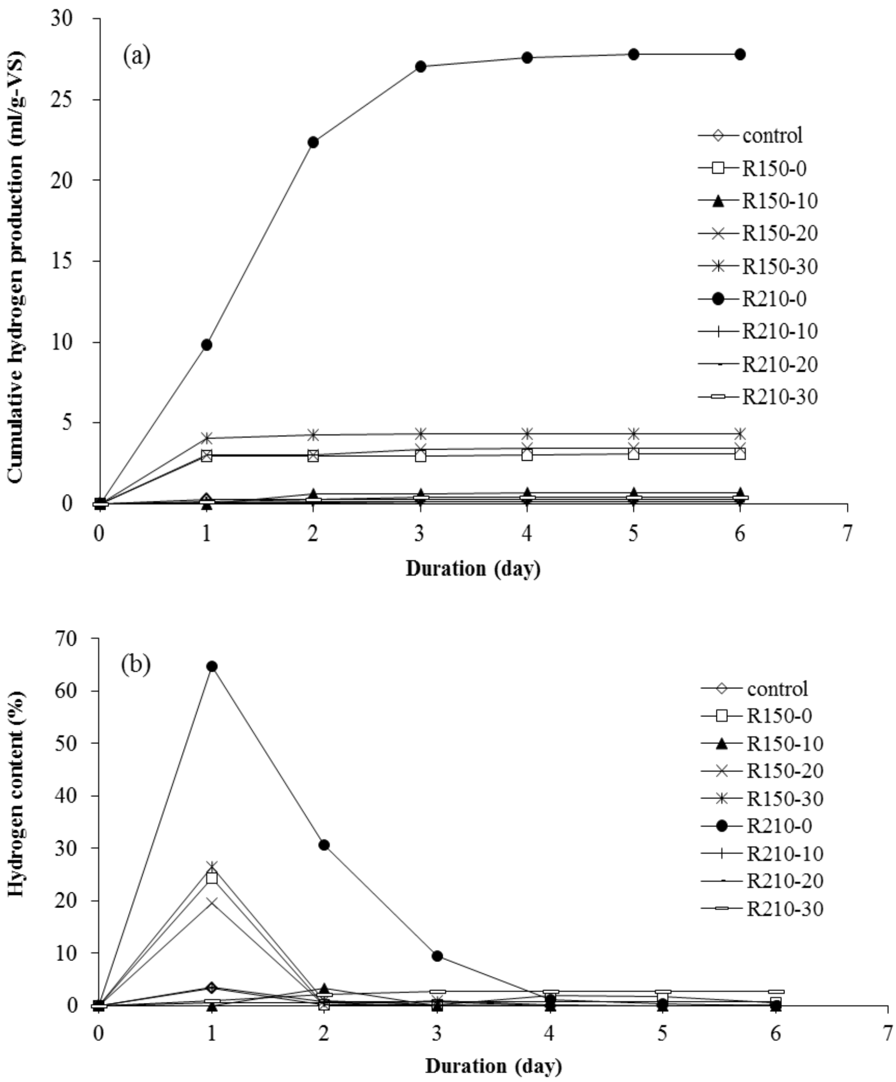

Figure 3.4 Biohydrogen production by using the resultant substrates from HTT pretreatment of rice straw: Cumulative H_2 production (a) and H_2 content in the produced biogas (b).

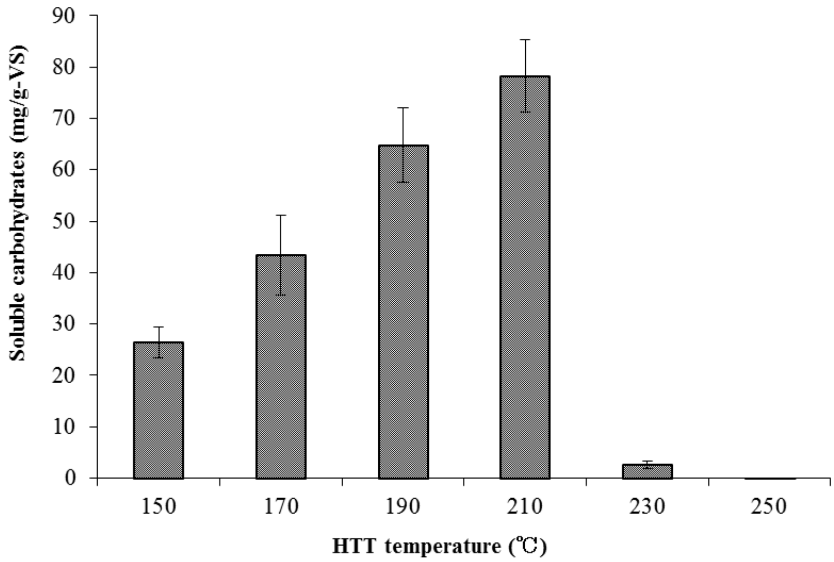

Figure 3.5 Soluble carbohydrates production from rice straw under different peak temperatures (150-250℃) of the HTT reactor with holding time of 0 min.

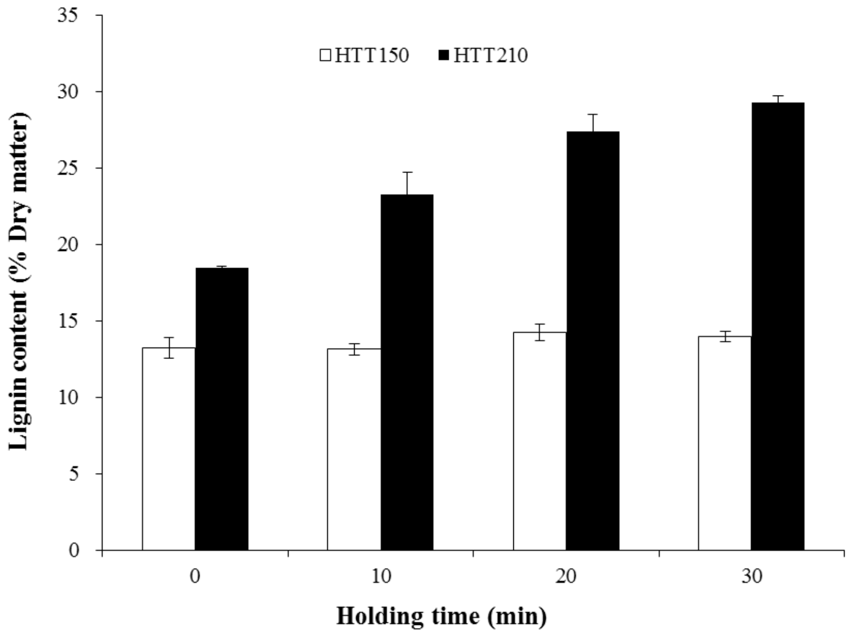

Figure 3.6 Variation of lignin content in rice straw after hydrothermal pretreatment under peak temperature of 150℃ (HTT150) and 210℃ (HTT210) for holding time of 0-30 min.

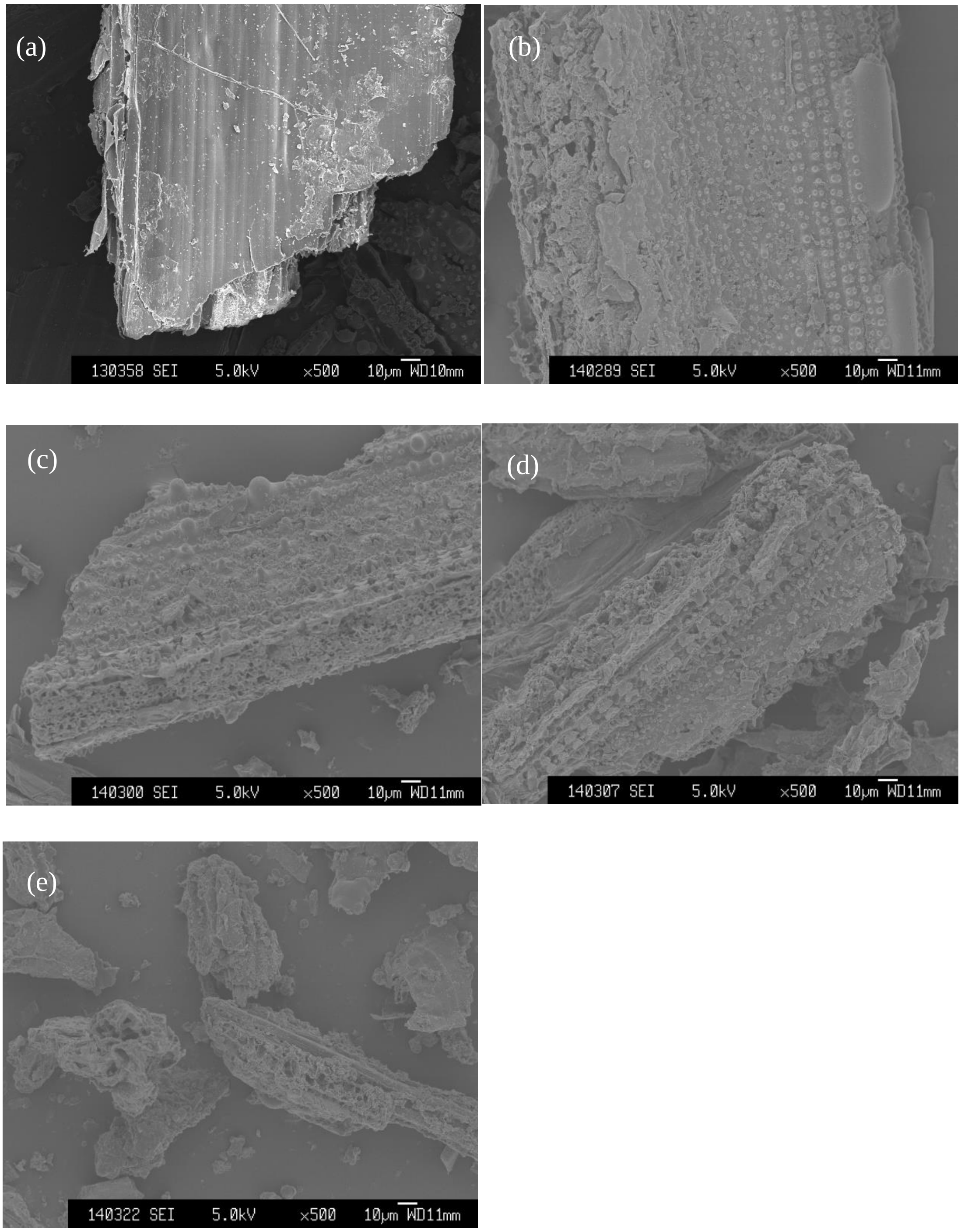

Figure 3.7 Morphological changes of rice straw particles before and after HTT pretreatment: raw rice straw (a), HTT150 for holding 0 min (b) and 30 min (c), and HTT210 for holding 0 min (d) and 30 min (e), respectively.

4. Comparative study of biogas production from rice straw pretreated by HTT: single-stage methane fermentation or two-stage hydrogen/methane fermentation

4.1. Introduction

In Chapter 3, the effect of determined peak temperature (150℃ and 210℃) and holding time (0-30 min) on the solubilization of rice straw at TS of 20% and then subsequent H_2 production from the resultant substrates was investigated. Although the HTT was proved to be a favorable pretreatment for hydrogen production, a large proportion of the total SCOD still hasn't been utilized for other purposes like methane fermentation. Therefore, it's not appropriate to compare and evaluate the energy input and output. And this evaluation was conducted in this chapter. The economic, social and environmental benefits from HTT with subsequent H_2/CH_4 productions were analyzed. More importantly, the performance of single-stage and two-stage fermentation was also evaluated.

4.2. Methods

4.2.1. Rice straw and seed sludge

Rice straw in this study was collected from a farm field in Tsukuba (Ibaraki, Japan), then air-dried and milled according to Section 2.2.1. The seed sludge for hydrogen fermentation was anaerobically digested sludge sampled from the Shimodate Sewage Treatment Center (Ibaraki, Japan). The seed sludge for methane fermentation was obtained from our preliminary acclimation experiments, which has been adapted to rice straw digestion under anaerobic condition. The physical and chemical characteristics of rice straw and seed sludge are shown in Table 4.1.

4.2.2. HTT procedure

HTT pretreatment was conducted in an enclosed stainless steel reactor with working volume of 200 ml. The reactor was loaded with a mixture of 100 ml distilled water and the prepared straw with total solid (TS) content being adjusted to around 20%. Two different peak temperatures (150℃ and 210℃, labeled as HTT150 and HTT210) were applied in HTT experiments at different holding time, i.e. 0min, 10min, 20min, and 30min, respectively. The temperature variation in the HTT reactor under each operation condition was described in Chapter 3.

4.2.3. Single-stage methane fermentation

Batch single-stage methane fermentation experiments were carried out in 100 ml glass bottles. After pretreated under above mentioned conditions (different peak temperature and holding time), the resultant substrate, a mixture of treated rice straw and soluble substances (hydrolysate) produced during the HTT process, was first mixed homogeneously and sampled, and then inoculated with seed sludge (inoculation ratio around 30% based on TS values of the inoculum and the initial fermentation substrate). The initial pH of each reactor was adjusted around 7.0. Their final volume was made up to 80 ml by using deionized water before fermentation. All the fermentation bottles (Table 4.2) were sealed with silica gel stoppers, and placed in a thermostat controlled at 35±1℃ after their headspace being flushed with nitrogen gas.

4.2.4. Two-stage hydrogen/methane fermentation

The first stage (for H_2 production) was carried out in 250 ml glass bottles. The operation process was the same as the previous batch hydrogen fermentation experiment in Section 2.2.3. After 6 days hydrogen fermentation, the hydrolysate from hydrogen fermentation was inoculated with seed sludge (inoculation ratio around 30% based on TS values of the inoculum and the initial fermentation substrate) and were further used to produce methane in 100 ml glass bottles (the second stage). The initial pH of each reactor was adjusted around

7.0. Their final volume was made up to 80 ml by using deionized water before fermentation. All the fermentation bottles (Table 4.2) were sealed with silica gel stoppers, and placed in a thermostat controlled at 35±1℃ after their headspace being flushed with nitrogen gas.

4.2.5. Analytical methods

Total organic carbon (TOC) and total organic nitrogen (TON) for solid sample was measured by means of Perkin-Elmer 2400 CHN Elemental Analyzer (Perkin-Elmer, Japan). Soluble TOC analysis was conducted with a TOC-V analyzer (Shimadzu, Japan). TS, VS, soluble carbohydrates, volatile fatty acids (VFAs), pH values, biogas analysis were determined according to the methods used in Sections 2.2.4 and 3.2.4.

4.3. Results and discussion

4.3.1. Biogas and methane production

(1) Single-stage anaerobic digestion

Figs. 4.1a and 4.1b described cumulative biogas and daily methane production by using the resultant substrates from HTT of rice straw for single-stage anaerobic digestion during 45 days` trials. The HTT150 treated substrate showed higher methane potential than HTT210. HTT150 for 20 min is optimum condition for subsequent methane production, with the highest methane yield of 149 ml/g-VS being obtained. Compared to the control, the highest increase in methane yield (up to 23%) was found under this condition. It was observed that 80% of total biogas was completed under HTT150 conditions during the first 30 days, however little biogas was detected from HTT210 pretreated rice straw during the first 20 days and the corresponding methane yields varied from 31 to 76 ml/g-VS. In addition, compared with the control, HTT210 seems to have negative influence on methane production and fermentation period. The reason probably attributes to the formation of phenolic compounds and furan derivatives under high HTT temperatures. The undesirable substances produced not only represent a loss of fermentable sugars, but also inhibit the activity of Bacteria and Archaea (Hernandez et al., 2008; Negro et al., 2003). Generally, the temperature above 200℃ is considered as the threshold which could limit methane production from the resultant hydrolyte (Hendriks and Zeeman, 2009; Neyens and Baeyens, 2003; Teghammar et al., 2010). On the other hand, Chandra et al. (2012) suggested addition of sodium hydroxide into the fermentor is important for enhanced methane yield (132.7 ml/g-VS) from hydrothermal pretreated rice straw at high temperature (200℃ for 10min).

Table 4.4 lists the maximum methane yields from crop residues and their operation conditions. Compared with other HTT conditions (mainly including temperature and reaction time), HTT pretreatment (at 150℃ for 20 min in this study) possesses the advantages like no sodium hydroxide addition, no steam explosion pretreatment and relatively lower energy input.

(2) Two-stage anaerobic digestion

Figs. 5.2a and 5.2b illustrate the cumulative biogas and daily methane production by using the resultant substrates from HTT pretreated rice straw for two-stage anaerobic digestion in the 25 days` methane producing stage. Compared with single-stage anaerobic digestion, the whole digestion period was shortened from 45 days to 31days (6 days hydrogen fermentation and 25 days methane fermentation). The methane production peaked around day 6 in R150-0, R150-10, R150-20, R150-30. However, the peak occurred around day 14 in R210-0, R210-10, R210-20, R210-30. Similarly, the maximum methane yield 162 ml/g-VS was also obtained at HTT150 for 20 min, which is 8% higher than R150-20 in the single-stage anaerobic digestion.

In this study, the methane content in the methane producing stage of the two-stage anaerobic digestion was found to be higher than that in the single stage (Table 4.3). Compared to the optimum conditions (HTT150 for 20 min) in the two types of anaerobic digestion, the methane content was enhanced from 46.3% in the single stage to 53% in the methane producing stage of two-stage anaerobic digestion. When compared to the control (163 ml/g-VS of biogas yield), HTT 210 for 0-30 min achieved 7-52% increase in biogas yields (175-248 ml/g-VS).

Compared to the single-stage fermentation, the two-stage process has been proved as a possible solution to improve the energy recovery and efficiency (Demirel and Yenigün, 2002). The result from this study also indicated that the two-stage fermentation had a higher methane production potential than the conventional single-stage anaerobic digestion (Table 4.3).

4.3.2. Efficiency in organic matter degradation

As seen from Table 4.3, the maximum methane yield 149 ml/g-VS and biogas yield 321 ml/g-VS were achieved in R150-20 in the single-stage anaerobic digestion, accompanied by TS and VS removals of 53% and 62.2%, respectively. Compared to the single-stage anaerobic digestion, R150-20 showed higher methane yield (162 ml/g-VS) with 41.1% of VS removal in the second stage (CH_4) of two-stage anaerobic digestion.

Figure 4.3 describes the initial and final conditions for methane production from HTT pretreated rice straw in the reactors. In the single-stage anaerobic digestion, correspondingly to high methane yields at HTT150, the concentration of acetic acid decreased dramatically after anaerobic digestion. On the contrary, a great amount of acetic acid was still remained in the reactor after fermentation with correspondingly low methane yield for HTT210, and its final VFAs concentration was much higher than the initial value. However, in the two-stage anaerobic digestion, after hydrogen producing stage, little VFAs accumulation was detected in the reactors for HTT210. This phenomenon indicated that the methane producing phase in the two-stage process had better buffer capacity and thus better VFAs utilization of methanogens than the conventional single-stage anaerobic digestion.

Figure 4.5 depicts the variation of soluble TOC during methane producing stage of the two-stage anaerobic digestion. Although the initial TOC concentration of HTT150 was lower than that of HTT210, the soluble TOC concentrations decreased dramatically from Day 5 for HTT150. While after 5 days' hydrolysis a lag phase of several days appeared for soluble TOC of HTT210. It was probably due to the low pH occurred after 5 days' hydrolysis, which is not appropriate for methane fermentation (Figure 4.4). It has been reported that pH has a significant effect on the growth of microorganisms in anaerobic digestion. Low pH affects the activity of methanogens as well as metabolic pathways. In order to maintain the stability of the anaerobic digestion, keeping the pH at the optimum conditions is important. The pHs in almost all the reactors of HTT150 were determined to be above 6.5 during anaerobic digestion. And their methane yields were higher than those of HTT210.

4.4. Summary

In this chapter, the effluent of hydrogen fermentation of HTT pretreated rice straw substrate was used for methane fermentation. The feasibility of the two-stage anaerobic digestion was discussed. In addition, the conventional single-stage anaerobic digestion was also conducted for evaluation and comparison of the performance of the single-stage and two-stage fermentation. The results can be summarized as: (1) The two-stage fermentation showed a higher methane production potential than the conventional single-stage anaerobic digestion. Compared with the single-stage anaerobic digestion, the whole fermentation period of two-stage anaerobic digestion was shortened from 45 days to 31 days; (2) HTT210 had a negative influence on the methane production and fermentation period in the single-stage anaerobic digestion. HTT150 showed higher methane production potential than HTT210 in both single-stage and two-stage anaerobic digestion; (3) The maximum methane yield 162 ml/g-VS was obtained from HTT150 for 20min in the methane producing stage of two-stage anaerobic digestion.

Table 4.1 Physical and chemical characteristics of rice straw and seed sludge used in the experiments.

Items	Unit	Rice straw	Sludge for hydrogen fermentation	Sludge for methane fermentation
pH	-	N.D.	7.31	7.52
Total solid (TS)	%	90.11	1.14	1.16
Volatile solid/Total solid (VS/TS)	%	79.37	68.47	69.02
Total organic carbon (TOC)	% TS	35.18	32.62	33.07
Total organic nitrogen (TON)	% TS	0.66	4.82	5.53
Soluble total organic carbon (STOC)	mg/l	N.D.	173	190

N.D., no determination.

Table 4.2 Hydrothermal treatment (HTT) and subsequent anaerobic digestion (AD) conditions applied in this study.

Reactors	HTT pretreatment conditions		Single-stage AD		Two-stage AD			
			Initial solid contents for CH_4 fermentation		Initial solid contents for H_2 fermentation		Initial solid contents for CH_4 fermentation	
	Peak temperature (℃)	Holding time (min)	TS (%)	VS/TS (%)	TS (%)	VS/TS (%)	TS (%)	VS/TS (%)
Control	-	-	4.52	77.65	5.84	78.40	3.00	72.33
R150-0	150	0	5.25	78.36	5.75	78.08	2.91	72.00
R150-10	150	10	5.80	78.34	5.71	78.56	2.98	72.81
R150-20	150	20	4.69	78.00	5.75	78.25	3.02	71.51
R150-30	150	30	5.46	77.92	5.74	77.99	2.99	71.18
R210-0	210	0	4.38	75.79	5.45	76.86	3.09	69.70
R210-10	210	10	5.13	73.29	4.98	74.70	2.88	70.14
R210-20	210	20	6.31	73.75	4.96	74.67	2.97	71.01
R210-30	210	30	4.08	73.28	4.86	74.27	2.82	69.50

AD: anaerobic digestion. Control: no pretreatment by HTT.

Initial pH of each fermentation reactor was adjusted to be around 7.0.

Table 4.3 Average performance for methane production of HTT pretreated rice straw in the reactors

Reactors	Performance for single-stage AD					Performance for methane producing stage of two-stage AD				
	TS removal (%)	VS removal (%)	Methane yield (ml/g-VS)	Biogas yield (ml/g-VS)	Methane content (%)	TS removal (%)	VS removal (%)	Methane yield (ml/g-VS)	Biogas yield (ml/g-VS)	Methane content (%)
Control	49.6	59.4	121	286	42.3	29.8	39.2	83	163	50.9
R150-0	56.9	64.9	134	300	44.7	28.7	40.0	140	269	52.0
R150-10	42.3	52.8	119	266	44.7	30.7	43.4	134	253	53.0
R150-20	53.0	62.2	149	321	46.4	29.7	41.1	162	293	55.3
R150-30	28.6	37.3	137	319	42.9	31.7	43.1	148	272	54.4
R210-0	42.6	50.0	41	135	30.4	31.3	41.6	131	248	52.8
R210-10	55.5	61.2	76	172	44.2	22.6	31.4	101	200	50.5
R210-20	26.4	35.2	31	96	32.3	22.8	32.0	71	175	40.6
R210-30	34.9	44.9	61	162	37.7	20.0	28.8	94	205	45.9

Table 4.4 Maximum methane yields from crop residues after HTT pretreatment by batch anaerobic digestion tests.

Crop residues	Inoculum	Methane yield (ml/g-VS)	Biogas yield (ml/g-VS)	Pretreatment method	Methane Fermentation conditions			Reference
					Temperature (℃)	HRT (d)	Initial pH	
Rice straw	AD sludge	149	321	HTT at 150℃ for holding 20 min	35	45	7.0	This work
Rice straw	AD sludge	121	286	No HTT pretreatment	35	45	7.0	This work
Rice straw	AD sludge	133	316	200℃ for 10 min	37	40	---*	Chandra et al., 2012
Wheat straw	AD sludge	296	---*	200℃ for 5 min+steam explosion	35.1	45	---*	Ferreira et al., 2014
Wheat straw	Digested manure	396	---*	80℃ for 6 min+180℃ for 15 min+190℃ for 3min	55	60	---*	Kaparaju et al., 2009.
Olive husks(mixed with olive mill wastewater and dairy wastewater)	AD sludge	---*	144	134℃ for 20 min	37	30	7.5	Gianico et al., 2013
Barley straw	Digestates of agricultural waste and municipal sewage sludge	251	---*	190℃for 30 min	38	34	---*	Schumacher et al.,2014

AD, anaerobic digested. * No data in the literature.

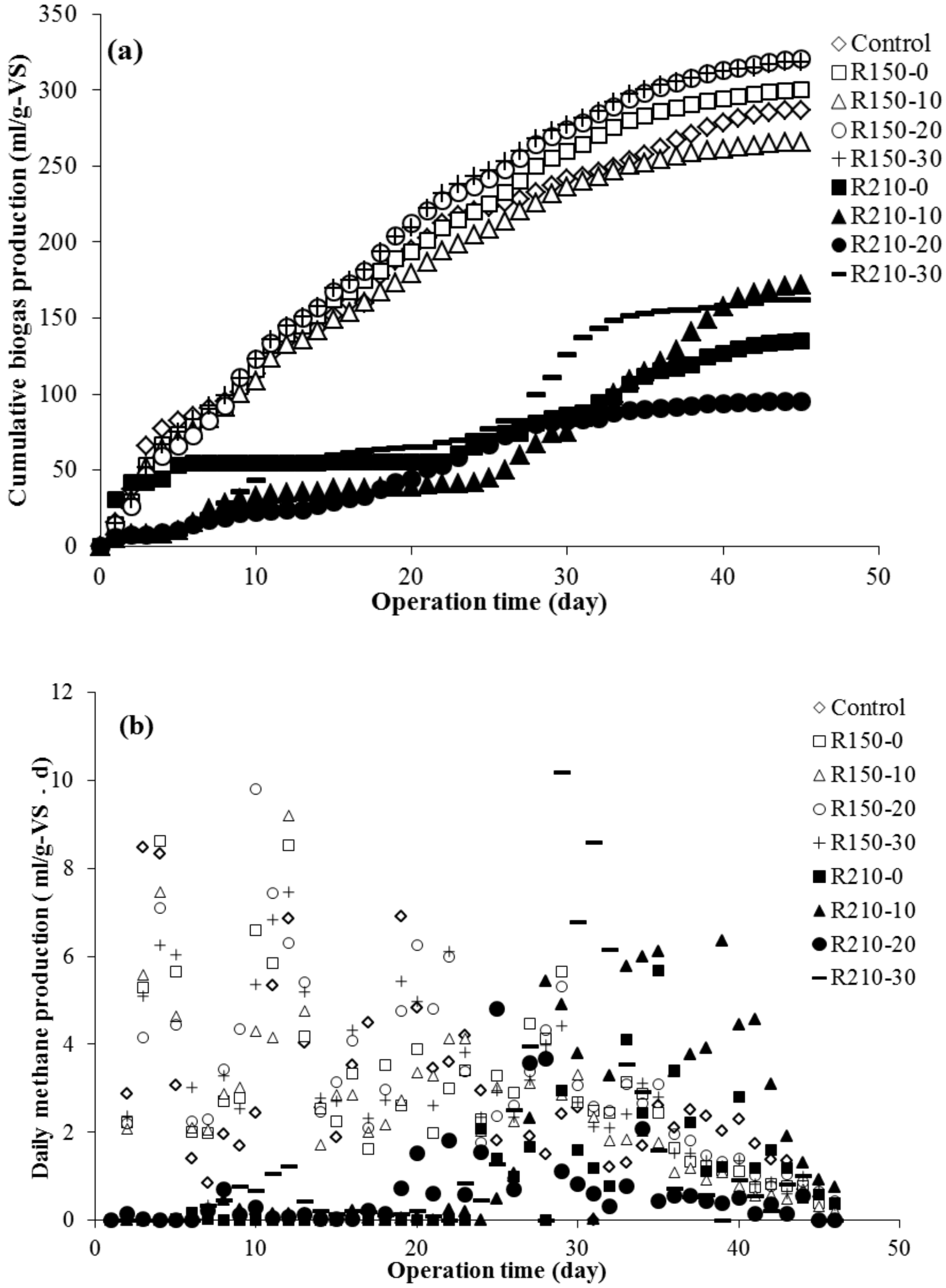

Figure 4.1 Biogas production by using the resultant substrates from HTT pretreatment of rice straw for single-stage anaerobic digestion: cumulative biogas production (a) and daily methane production (b).

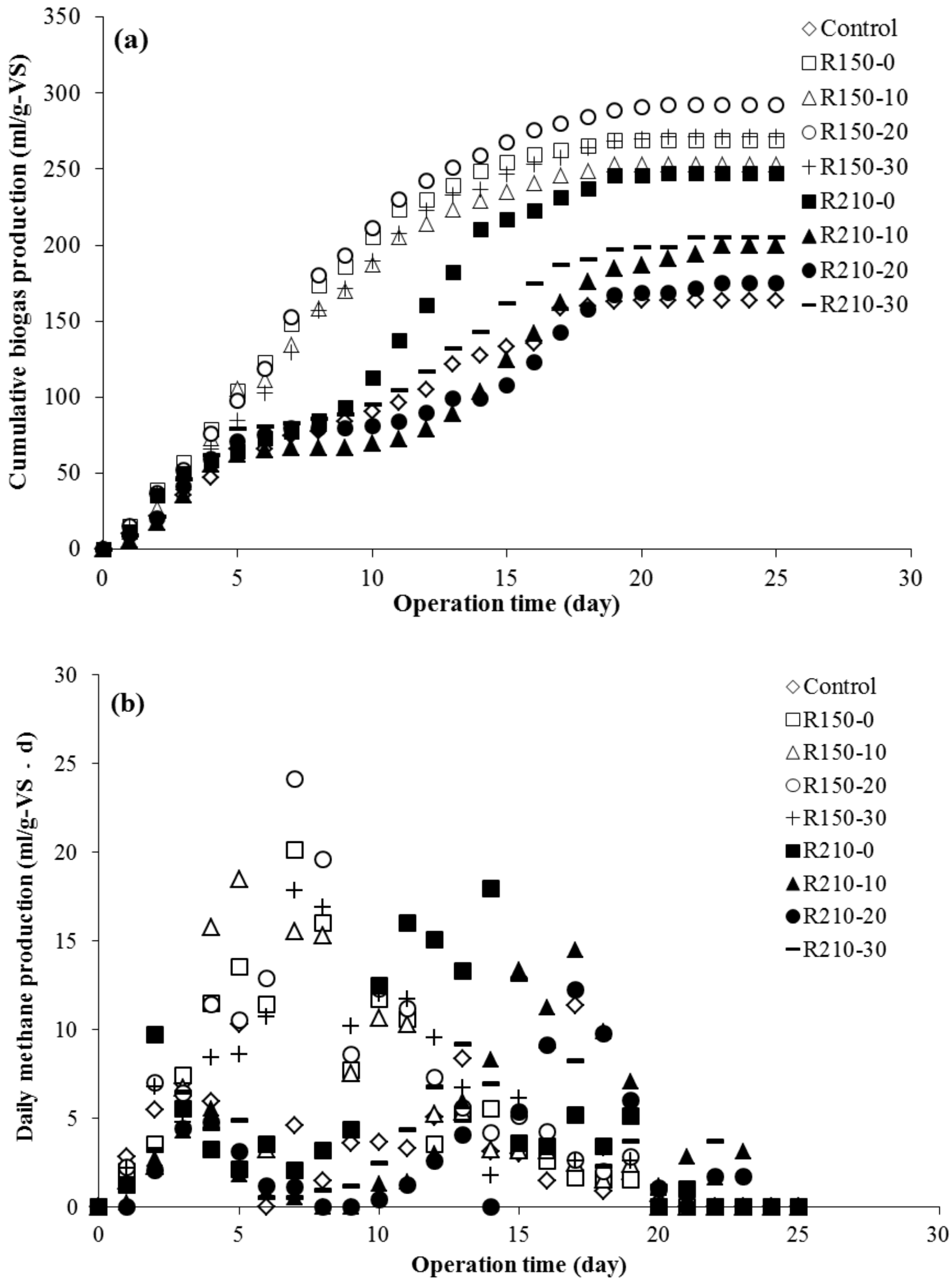

Figure 4.2 Biogas production from the effluent of hydrogen fermentation of HTT pretreated rice straw during the second stage (CH_4 production) of two-stage anaerobic digestion: cumulative biogas production (a) and daily methane production (b).

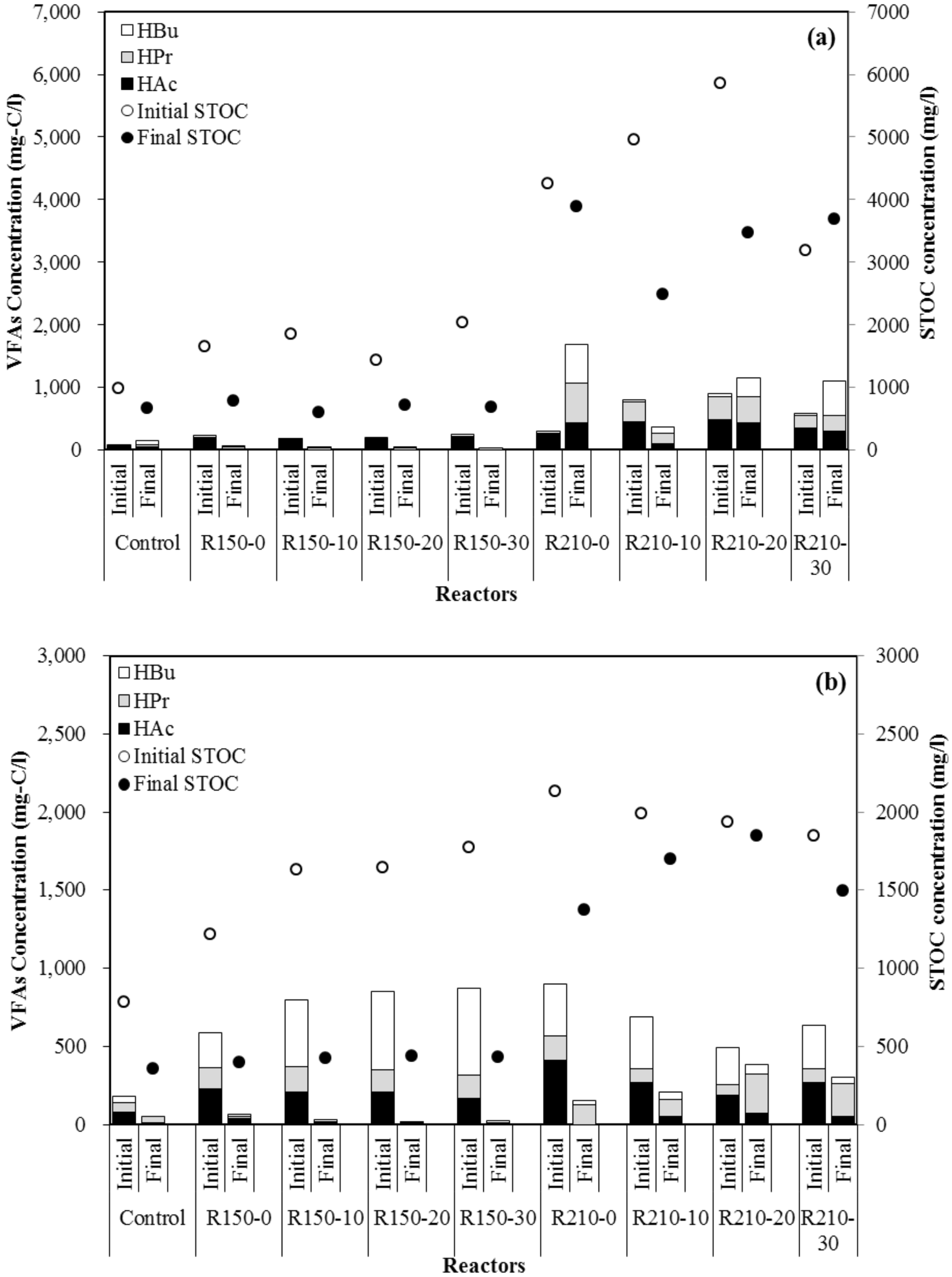

Figure 4.3 Initial and final conditions for methane production of HTT pretreated rice straw in the reactors: the single-stage anaerobic digestion (a) and CH_4 stage in the two-stage anaerobic digestion (b). HAc: acetic acid; HPr: propionic acid; HBu: butyric acid.

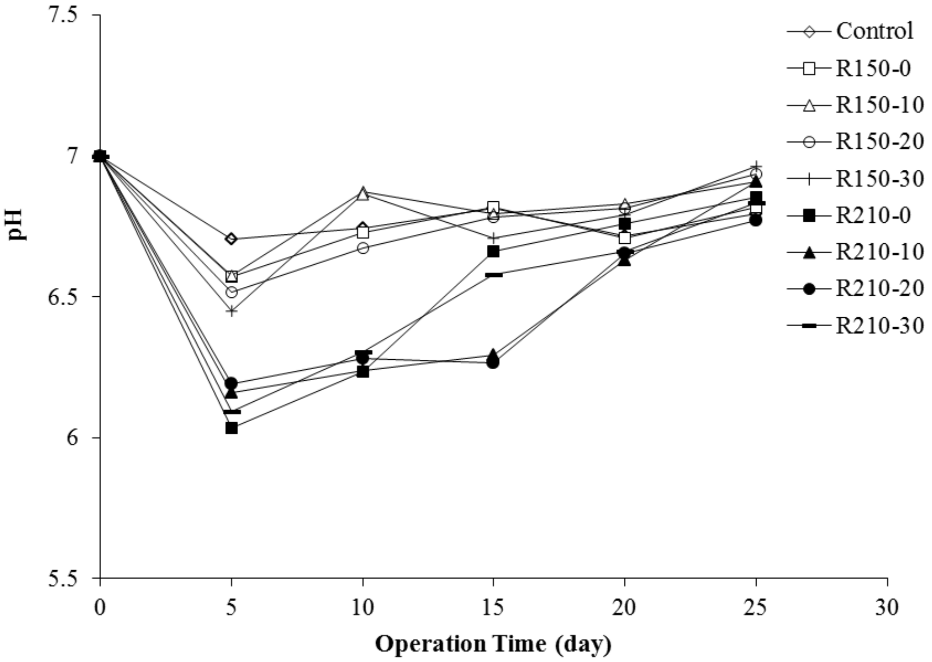

Figure 4.4 Variation of pH during the methane producing stage of two-stage anaerobic digestion.

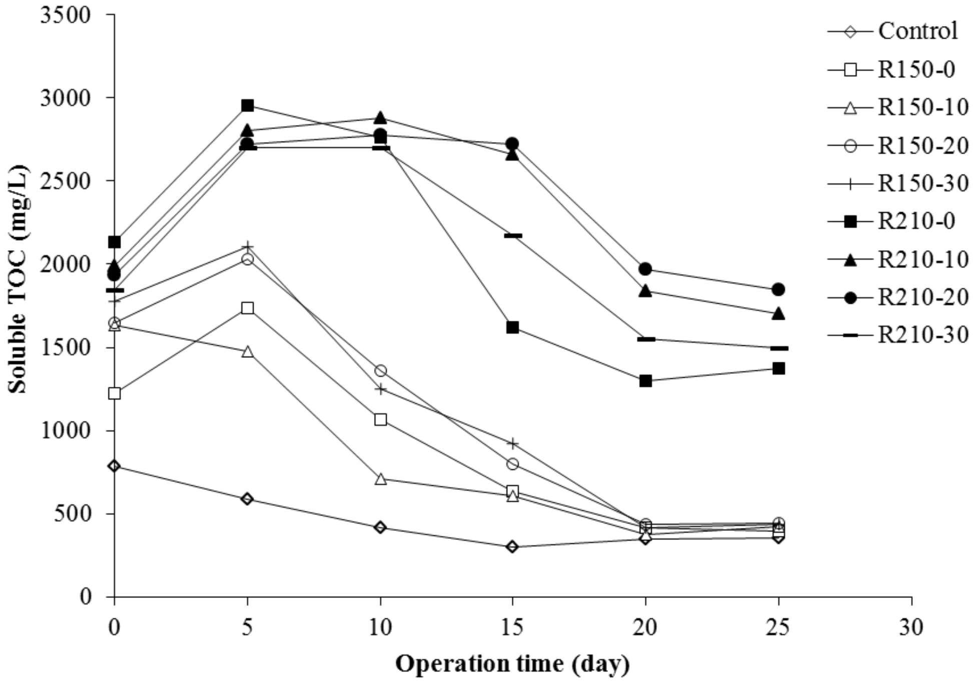

Figure 4.5 Variation of soluble TOC during the methane producing stage of two-stage anaerobic digestion

5. Conclusions and future research

5.1. Conclusions

Hydrothermal pretreatment of lignocellulosic biomass is promising for enhanced biogasification and re-utilization of lignocellosic materials. This study paid much attention to the major operational parameters of HTT which affect the hydrogen and methane yields in subsequent anaerobic digestion. Temperature is considered as the most important parameter in HTT pretreatment of biomass when enhanced hydrogen production and methanogenesis are taken into consideration. Fig. 5.1 shows the overview of this study.

In the preliminary experiments, the effect of different HTT conditions including temperature (190-290℃), holding time (1.5 min) and solid content (4-20%) on rice straw pretreatment were investigated. In addition, batch experiments of mesophilic anaerobic digestion were also carried out to verify the enhancement on biogasification when hydrolyzed rice straw by HTT was used as feedstock. The results showed that higher temperature was effective for reduction of solid content. However, when temperature was above 230℃, SCOD did not increase significantly with the increase in temperature. Moreover, less soluble carbohydrates were obtained at higher temperatures (＞230℃). When considering high hydrogen production and energy recovery, moderate temperature (210℃) and high solid content (20%) was found to be favorable for biogasification.

In the followed-up study, the experiments on the effects of HTT pretreatment at two peak temperatures (150℃ and 210℃) with holding time of 0 - 30 min were designed and performed to investigate the solubiliztion of rice straw and subsequent biohydrogen production. HTT pretreatment at peak temperature of 210℃ could achieve the maximum amount of soluble carbohydrates (80 mg/g-VS) and biohydrogen yield (28 mg/g-VS) when the holding time was 0 min. The amount of soluble carbohydrates produced from rice straw during HTT treatment was found to have strongly positive correlation with the H_2 yield from subsequent H_2 fermentation.

In addition, the effluent from hydrogen fermentation of HTT pretreated rice straw substrate was used for methane fermentation. The feasibility of the two-stage anaerobic digestion (i.e. the first stage for H_2 production and the second stage for CH_4 production) was explored with the conventional single-stage anaerobic digestion as control in order to evaluate and compare the performance of the single-stage and two-stage fermentation. The two-stage process showed a higher methane production potential than the conventional single-stage. Compared to the single-stage process, the two-stage process achieved a shortened fermentation period from 45 to 31 days. When compared to the control, HTT210 seemed to have negative effect on the methane production potential in the single-stage process with methane yields varied from 31 to 76 ml/g-VS. HTT150 pretreated straw had a higher methane production potential than HTT210 in the single-stage and the two-stage processes. In addition, the maximum methane yield of 162ml/g-VS was obtained from HTT150 pretreated rice straw (for 20 min) in the methane producing stage of the two-stage process.

5.2. Future prospects and works

Although much effort has been tried to enhance the biogasificarion from rice straw, up to now, future research works are still demanding in order to realize its practical application. In this study, rice straw was utilized for biogasification after hydrothermal pretreatment. The results of this work can provide useful information for the combined application of HTT pretreatment with fast biohydrogen fermentation technique of crop straws. The present results show that it’s feasible to use HTT pretreated rice straw for subsequent H_2/CH_4 production in practice. In addition, the two-stage process may be a possible applicable method for further enhancing biogasification from HTT pretreated rice straw substrate.

Based on the results from this study, still, some aspects are pending for the utilization of HTT pretreatment in practice: (1) The mechanisms of enhanced methane production in the two-stage process remain unknown. (2) The economic, social and environmental benefits from HTT with subsequent H_2/CH_4 productions should be systematically assessed. (3) How to reclaim solid residue of rice straw after HTT pretreatment is also necessary and important.

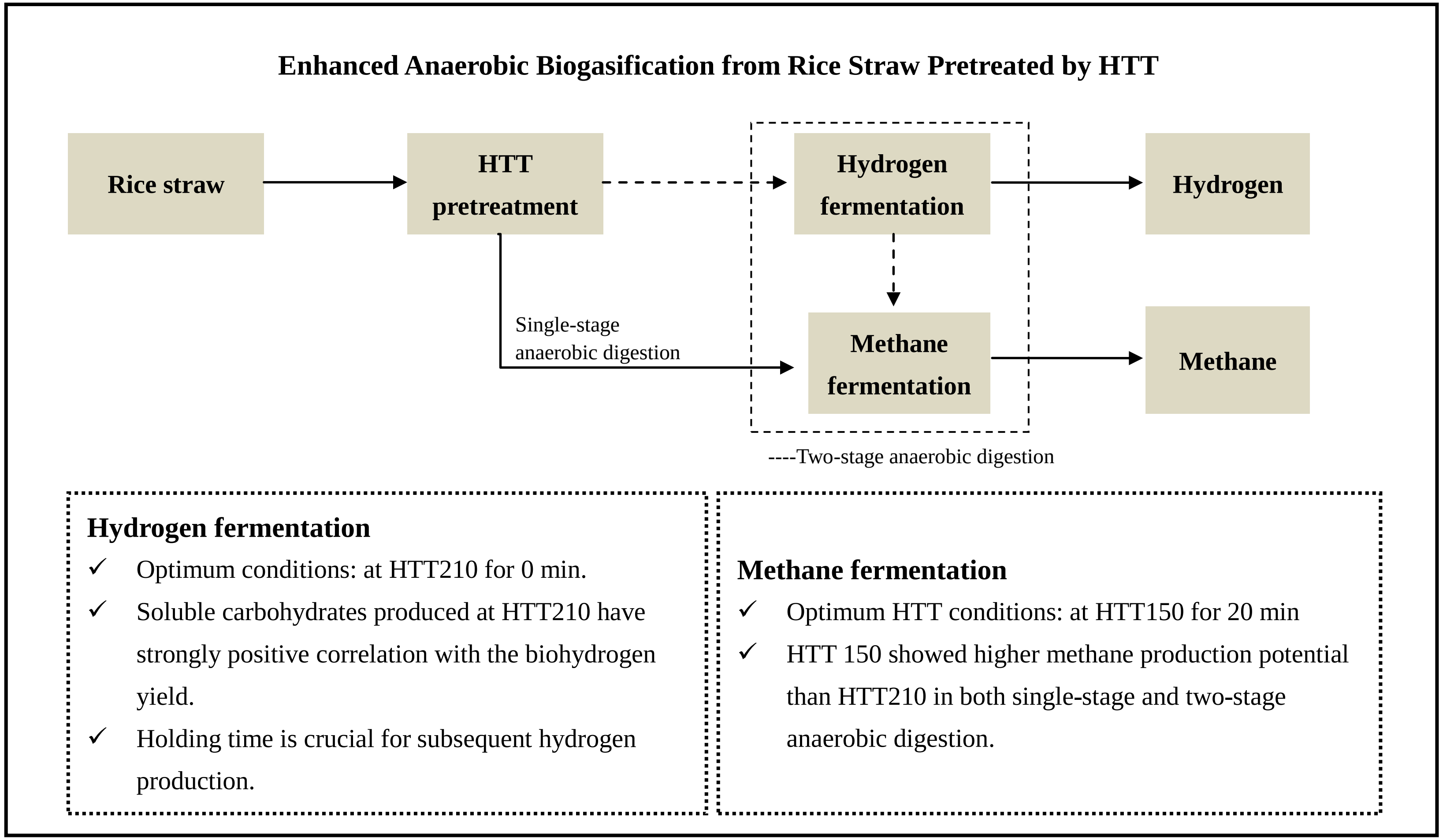

Figure 5.1 Overview of this study

References

Adl, M., Sheng, K., Gharibi, A., 2012. Technical assessment of bioenergy recovery from cotton stalks through anaerobic digestion process and the effects of inexpensive pretreatments. Appl. Energ. 93, 251-260.

APHA, 2005. Standard Methods for the Examination of Water and Wastewater, 21st Edition. American Public Health Association/ American Water Works Association/ /Water Environment Federation, Washington, DC.

Akhtar, J., Amin, N.A.S., 2011. A review on process conditions for optimum bio-oil yield in hydrothermal liquefaction of biomass. Renew. Sust. Energ. Rev. 15, 1615-1624.

Allen, S.G., Schulman, D., Laser, M., Lichwa, J., Antal Jr., M.J., Lynd, L.R, 2001. A comparison between hot liquid water and steam fractionation of corn fiber. Ind. Eng. Chem. Res. 40, 2934-2941.

Antonopoulou, G., Lyberatos, G., 2013. Effect of pretreatment of sweet sorghum biomass on methane generation. Waste Biomass Valor. 4, 583-591.

Appels, L., Degrève, J., Van der Bruggen, B., Van Impe, J., Dewil, R., 2010. Influence of low temperature thermal pre-treatment on sludge solubilisation, heavy metal release and anaerobic digestion, Bioresource Technol. 101, 5743-5748.

Asghari, F.S., Yoshida, H., 2006. Acid catalyzed production of 5-hydroxymethyl furfural from D-fructose in subcritical water. Ind. Eng. Chem. Res. 45, 2163-2173.

Bayr, S., Kaparaju, P., Rintala, J., 2013. Screening pretreatment methods to enhance thermophilic anaerobic digestion of pulp and paper mill wastewater treatment secondary sludge. Chem. Eng. J. 223, 479-486.

Benjamin, M.M., Woods, S.L., Ferguson, J.F., 1984. Anaerobic toxicity and biodegradability of pulp-mill waste constituents. Water Res. 18, 601-607.

Berlin, A., Balakshin, M., Gilkes, N., Kadla, J., Maximenko, V., Kubo, S., Saddler, J., 2006.

Inhibition of cellulase, xylanase and beta-glucosidase activities by softwood lignin preparations. J. Biotechnol. 125, 198-209.

Bobleter, O., 1994. Hydrothermal degradation of polymers derived from plants. Prog. Polym. Sci. 19, 797-841.

Bougrier, C., Delgenes, J.P., Carrere, H., 2008. Effects of thermal treatments on five different waste activated sludge samples solubilisation, physical properties and anaerobic digestion. Chem. Eng. J. 139, 236-244.

Bruni, E., Jensen, A.P., Angelidaki, I., 2010. Steam treatment of digested biofibers for increasing biogas production. Bioresource Technol. 101, 7668-7671.

Budde, J., Heiermann, M., Quiñones, T. S., Plöchl, M., 2014. Effects of thermobarical pretreatment of cattle waste as feedstock for anaerobic digestion. Waste Manage. 34, 522-529.

Cao, G., Ren, N., Wang, A., Guo, W., Xu, J., Liu, B., 2010. Effect of lignocellulose-derived inhibitors on growth and hydrogen production by *Thermoanaerobacterium thermosaccharolyticum* W16. Int. J. Hydrogen Energy 35, 13475-1348.

Chandra, R., Takeuchi, H., Hasegawa, T., 2012. Hydrothermal pretreatment of rice straw biomass: A potential and promising method for enhanced methane production. Appl. Energ. 94, 129-140.

Costa, A.G., Pinheiro, G.C., Pinheiro, F.G.C., Dos Santos, A.B., Santaella, S.T., Leitão, R.C., 2013. Pretreatment strategies to improve anaerobic biodegradability and methane production potential of the palm oil mesocarp fibre. Chem. Eng. J. 230, 158-165.

Costa, A.G., Pinheiro, G.C., Pinheiro, F.G.C., Dos Santos, A.B., Santaella, S.T., Leitão, R.C., 2014. The use of thermochemical pretreatments to improve the anaerobic biodegradability and biochemical methane potential of the sugarcane bagasse. Chem. Eng. J. 248, 363-372.

Daorattanachai, P., Viriya-empikul, N., Laosiripojana, N., Faungnawakij, K., 2013. Effects of

Kraft lignin on hydrolysis/dehydration of sugars, cellulosic and lignocellulosic biomass under hot compressed water. Bioresource Technol. 144, 504-512.

Darwish, G.A.M.A., Bakr, A.A., Abdallah, M.M.F., 2012. Nutritional value upgrading of maize stalk by using Pleurotus ostreatus and Saccharomyces cerevisiae in solid state fermentation. Ann. Agric. Sci. 57, 47-51.

Delmer, D.P.; Amor, Y. Cellulose biosynthesis. Plant Cell 1995, 7, 987-1000.

Demirel, B., Yenigün, O., 2002. Two-phase anaerobic digestion processes: a review. J. Chem. Technol. Biot. 77, 743-755.

Di Girolamo, G., Grigatti, M., Barbanti, L., Angelidaki, I., 2013. Effects of hydrothermal pre-treatments on Giant reed (*Arundo donax*) methane yield. Bioresource Technol. 147, 152-159.

Donoso-Bravo, A., Pérez-Elvira, S., Aymerich, E., Fdz-Polanco, F., 2011. Assessment of the influence of thermal pre-treatment time on the macromolecular composition and anaerobic biodegradability of sewage sludge. Bioresource Technol. 102, 660-666.

Fan, Y., Zhang, Y., Zhang, S., Hou, H., Ren, B., 2006. Efficient conversion of wheat straw wastes into biohydrogen gas by cow dung compost. Bioresource Technol. 97, 500-505.

FAOSTAT, 2009. Food and Agricultural Organization of United Nations, Statistical Databases.

Fernández-Cegrí, V., De la Rubia, M. Á., Rubia, D.L., Raposo, F., Borja, R., 2012. Effect of hydrothermal pretreatment of sunflower oil cake on biomethane potential focusing on fibre composition. Bioresource Technol. 123, 424-429.

Fernandes, T.V., Klaasse Bos, G.J., Zeeman, G., Sanders, J.P.M., Van Lier, J.B., 2009. Effects of thermo-chemical pre-treatment on anaerobic biodegradability and hydrolysis of lignocellulosic biomass, Bioresource Technol. 100, 2575-2579.

Wyman, C.E. Ethanol production from lignocellulosic biomass: review, in: C.E. Wyman (Ed.), Handbook on Bioethanol, Production and Utilization, Taylor & Francis, Washington, DC, 1996.

Ferreira, L.C., Donoso-Bravo, A., Nilsen, P.J., Fdz-Polanco, F., Pérez-Elvira, S.I., 2013. Influence of thermal pretreatment on the biochemical methane potential of wheat straw. Bioresource Technol. 143, 251-257.

Ferreira, L.C., Nilsen, P.J., Fdz-Polanco, F., Pérez-Elvira, S.I., 2014. Biomethane potential of wheat straw: Influence of particle size, water impregnation and thermal hydrolysis. Chem. Eng. J. 242, 254-259.

Gao, Y., Wang, X., Yang, H., Chen, H., 2012. Characterization of products from hydrothermal treatments of cellulose. Energy 42, 457-465.

Garrote, G., Domínguez, H., Parajó, J.C., 1999. Hydrothermal processing of lignocellulosic materials. Holz. Roh. Werkst. 57, 191-202.

Gianico, A., Braguglia, C.M., Mescia, D., Mininni, G., 2013. Ultrasonic and thermal pretreatments to enhance the anaerobic bioconversion of olive husks. Bioresource Technol. 147, 623-626.

Gossett, J.M., Stuckey, D.C., Owen, W.F., McCarty, P.L., 1982. Heat treatment and anaerobic digestion of refuse. J. Env. Eng. Div. 108, 437-454.

Grabber, J.H., 2005. How do lignin composition, structure, and cross-linking affect degradability? A review of cell wall model studies. Crop Sci. 45,820-831.

Greenhalf, C.E., Nowakowski, D.J., Bridgwater, A.V., Titiloye, J., Yates, N., Riche, A., Shield, I., 2012. Thermochemical characterisation of straws and high yielding perennial grasses. Ind. Crop. Prod. 36, 449-459.

Guo, X., Trably, E., Latrille, E., Carrère, H., Steyer, J.P., 2010. Hydrogen production from agricultural waste by dark fermentation: A review. Int. J. Hydrogen Energy 35, 10660-10673.

Nallathambi Gunaseelan, V., 1997. Anaerobic digestion of biomass for methane production: a review. Biomass Bioenerg. 13, 83-114.

Hamelinck, C.N., van Hooijdonk, G., Faaij, A.P.C., 2005. Ethanol from lignocellulosic biomass: technoeconomic performance in short-, middle- and long-term. Biomass Bioenerg. 28, 384-410.

Hendriks, A.T.W.M., Zeeman, G., 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresource Technol. 100, 10-18.

Herbert, D., Philipps, P.J., Strange, R.E., 1971. Carbohydrate analysis. Methods Enzymol. 5B, 265-277.

Hernandez, J.E., Edyvean, R.G.J., 2008. Inhibition of biogas production and biodegradability by substituted phenolic compounds in anaerobic sludge. J. Hazard. Mater. 160, 20-28.

Hu, F., Ragauskas, A.J., 2011. Hydrothermal pretreatment of switchgrass, Ind. Eng. Chim. Res. 50, 4225-4230.

Ivanova, G., Rákhely, G., Kovács, K., 2009. Thermophilic biohydrogen production from energy plants by *Caldicellulosiruptor saccharolyticus* and comparison with related studies. Int. J. Hydrogen Energy 34, 3659-3670.

Jin F, Zhou Z, Enomoto H, Moriya T, Higashijima H, 2004. Conversion mechanism of cellulosic biomass to lactic acid in subcritical water and acid-base catalytic effect of subcritical water. Chem. Lett. 33, 126-127.

Kabyemela, B.M., Adschiri, T., Malaluan, R.M., Arai, K., 1997. Kinetics of glucose epimerization and decomposition in subcritical and supercritical water. Ind. Eng. Chem. Res. 36, 1552-1558.

Kaparaju, P., Serrano, M., Thomsen, A.B., Kongjan, P., Angelidaki, I., 2009. Bioethanol, biohydrogen and biogas production from wheat straw in a biorefinery concept. Bioresource Technol. 100, 2562-2568.

Khullar, E., Dien, B.S., Rausch, K.D., bleson, M.E., Singh, V., 2013. Effect of particle size on enzymatic hydrolysis of pretreated Miscanthus. Ind. Crop. Prod. 44, 11-17.

Kim, Y., Mosier, N.S., Ladisch, M.R., 2009. Enzymatic digestion of liquid hot water pretreated hybrid poplar. Biotechnol. Progr. 25, 340-348

Kristensen, J.B., Thygesen, L.G., Felby, C., Jørgensen, H., Elder, T., 2008. Cell-wall structural changes in wheat straw pretreated for bioethanol production. Biotechnol. Biofuels 1, 1-9.

Kumar, S., Kothari, U., Kong, L., Lee, Y.Y., Gupta, R.B., 2011. Hydrothermal pretreatment of switchgrass and corn stover for production of ethanol and carbon microspheres. Biomass Bioenerg. 35, 956-968.

Lamsal, B. P., Madl, R., Tsakpunidis, K., 2010. Comparison of Feedstock Pretreatment Performance and Its Effect on Soluble Sugar Availability. Bioenerg. Res.4, 193-200.

Laser, M., Schulman, D., Allen, S.G., Lichwa, J., Antal Jr., M.J., Lynd, L.R, 2002. A comparison of liquid hot water and steam pretreatments of sugar cane bagasse for bioconversion to ethanol. Bioresource Technol. 81, 33-44.

Li, H.-Q., Jiang, W., Jia, J.-X., Xu, J., 2014. pH pre-corrected liquid hot water pretreatment on corn stover with high hemicellulose recovery and low inhibitors formation. Bioresource Technol. 153, 292-299.

Lim, J. S., Manan, Z. A., Alwi S. R. W., Hashim, H., 2012. A review on utilisation of biomass from rice industry as a source of renewable energy. Renew. Sust. Energ. Rev. 16, 3084-3094.

Lin, C.-Y., Chen, H.-P. 2006. Sulfate effect on fermentative hydrogen production using anaerobic mixed microflora. Int. J. Hydrogen Energ. 31, 953-960.

Liu, C., Wyman, C.E., 2005. Partial flow of compressed-hot water through corn stover to enhance hemicellulose sugar recovery and enzymatic digestibility of cellulose. Bioresource Technol. 96, 1978-1985.

López González, L.M., Reyes, I. P., Dewulf, J., Budde, J., Heiermann, M., Vervaeren, H., 2014. Effect of liquid hot water pre-treatment on sugarcane press mud methane yield. Bioresource Technol. 169, 284-290.

Martins, S. I., Jongen, W. M., Van Boekel, M. A., 2000. A review of Maillard reaction in food and implications to kinetic modelling. Trends Food Sci. Tech. 11, 364-373.

Matsuo, Y., Yanagisawa, A., Yamashita, Y., 2013. A global energy outlook to 2035 with strategic considerations for Asia and Middle East energy supply and demand interdependencies. Energ. Strat. Rev. 2, 79-91.

Mendel, B., Kemp, A., Myers, D. K., 1954. A colorimetric micro-method for the determination of glucose. Biochem. J. 56, 639-646.

Merali, Z., Ho, J.D., Collins, S.R., Le Gall, G., Elliston, A., Kasper, A., Waldron, K.W. 2013. Characterization of cell wall components of wheat straw following hydrothermal pretreatment and fractionation. Bioresource Technol. 131, 226-234.

Miller, G.L., 1959. Use of dinitrosalicylic acid agent for determination of reducing sugar. Anal. Chem. 31, 426-428.

Minowa T, Fang Z, Ogi T, Varhegyi G, 1997. Liquefaction of cellulose in hot compressed water using sodium carbonate: products distribution at different reaction temperatures. J. Chem. Eng. Jpn. 30, 186-190.

Minowa T, Zhen F, Ogi T, 1998. Cellulose decomposition in hot-compressed water with alkali or nickel catalyst. J. Supercrit. Fluid. 13, 253-259.

Moniruzzaman, M., Ono, T., 2013. Separation and characterization of cellulose fibers from cypress wood treated with ionic liquid prior to laccase treatment. Bioresource Technol. 127, 132-137.

Moniza, P., Pereira, H., Quilhó, T., Carvalheiro, F., 2013. Characterisation and hydrothermal processing of corn straw towards the selective fractionation of hemicelluloses. Ind. Crop. Prod. 50, 145-153.

Monlau, F., Sambusiti, C., Barakat, A., Guo, X., Latrille, E., Trably, E., Steyer, J., Carrere, H., 2012. Predictive Models of Biohydrogen and Biomethane Production Based on the Compositional and Structural Features of Lignocellulosic Materials. Environ. Sci.

Technol. 46, 12217-12225.

Morohoshi, N., 1991. Chemical characterization of wood and its components. In: Hon, D.N.S, Shiraishi, N. (Eds.), Wood and Cellulosic Chemistry, New York, pp. 331-392.

Mosier, N.S., Hendrickson, R., Brewer, M., Ho, N., Sedlak, M., Dreshel, R., Welch, G., Dien, B.S., Aden, A., Ladisch, M.R., 2005a. Industrial scale-up of pH-controlled liquid hot water pretreatment of corn fiber for fuel ethanol production. Appl. Biochem. Biotech. 125, 77-97.

Mosier, N., Hendrickson, R., Ho, N., Sedlak, M., Ladisch, M.R., 2005b. Optimization of pH controlled liquid hot water pretreatment of corn stover. Bioresource Technol. 96, 1986-1993.

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y.Y., Holtzapple, M., Ladisch, M., 2005c. Features of promising technologies for pretreatment of lignocellulosic biomass, Bioresource Technol. 96, 673-686.

Nakhshiniev, B., Biddinikaa M.K., Gonzales, H.B., Sumidac, H., Yoshikawa, K., 2014. Evaluation of hydrothermal treatment in enhancing rice straw compost stability and maturity. Bioresource Technol. 151, 306-313.

Nallathambi Gunaseelan, V., 1997. Anaerobic digestion of biomass for methane production: a review. Biomass Bioenerg. 13, 83-114.

Nasirian, N., Almassi, M., Minaei, S., Widmann, R., 2011. Development of a method for biohydrogen production from wheat straw by dark fermentation. Int. J. Hydrogen Energy 36, 411-420.

Negro, M.J., Manzanares, P., Ballesteros, I., Oliva, J.M., Cabañas, A., Ballesteros, M., 2003. Hydrothermal pretreatment conditions to enhance ethanol production from poplar biomass. Appl. Biochem. Biotechnol. 108, 87-100.

Neyens, E., Baeyens, J., 2003. A review of thermal sludge pre-treatment processes to improve dewaterability. J. Hazard. Mater. 98, 51-67.

Nitsos, C.K., Konstantinos, A.M., Kostas, S.T., 2013. Optimisation of hydrothermal pretreatment of lignocellulosic biomass in the bioethanol production process. ChemSusChem 6, 110-122.

Op den Camp, H.J.M., Verhagen, F.J.M., Kivaisi, A.K., de Windt, F.E., Lubberding, H.J., Gijzen, H., Vogel, G.D., 1988. Effects of lignin on the anaerobic degradation of (ligno) cellulosic wastes by rumen microorganisms. Appl. Microbiol. Biot. 29, 408-412.

O-Thong, S., Boe, K., Angelidaki, I., 2012. Thermophilic anaerobic co-digestion of oil palm empty fruit bunches with palm oil mill effluent for efficient biogas production. Appl. Energ. 93, 648-54.

Overend, R.P., Chornet, E., 1987. Fractionation of lignocellulosics by steam-aqueous pretreatments. Philos. T. R. Soc. A 321, 526-533.

Pampulha, M.E., Lourero-Dias, M.C., 1989. Combined effect of acetic acid, pH, and ethanol on intracellular pH of fermenting yeast. Appl. Microb. Biotechnol. 31, 547-550.

Pérez, J.A., González, A., Oliva, J.M., Ballesteros, I., Manzanares, P., 2007. Effect of process variables on liquid hot water pretreatment of wheat straw for bioconversion to fuel-ethanol in a batch reactor. J. Chem. Technol. Biotechnol. 82, 929-938.

Peterson, A.A., Vogel, F., Lachance, R.P., Fröling, M., Antal Jr., M.J., Tester, J.W., 2008. Thermochemical biofuel production in hydrothermal media: A review of sub- and supercritical water technologies. Energy Environ. Sci. 1, 32-65.

Petersen, M.Ø., Larsena, J., Thomsen, M.H., 2009. Optimization of hydrothermal pretreatment of wheat straw for production of bioethanol at low water consumption without addition of chemicals. Biomass Bioenerg. 33, 834-840.

Qiao, W., Yan, X., Ye, J., Sun, Y., Wang, W., Zhang, Z., 2011. Evaluation of biogas production from different biomass wastes with/without hydrothermal pretreatment. Renew. Energ. 36, 3313-3318.

Quéméneur, M., Bittel, M., Trably, E., Dumas, C., Fourage, L., Ravot, G., Steyer, J., Carre`re, H., 2012. Effect of enzyme addition on fermentative hydrogen production from wheat straw. Int. J. Hydrogen Energy 37, 10639-10647.

Rasmussen, H., Sørensen, H.R., Meyer, A.S., 2014. Formation of degradation compounds from lignocellulosic biomass in the biorefinery: sugar reaction mechanisms. Carbohyd. Res. 385, 45-57.

REN21 (Renewable Energy Policy Network for the 21st Century). Renewables 2014 Global Status Report. http://www.ren21.net/REN21Activities/GlobalStatusReport.aspx (Accessed September 2, 2014)

Ren, N., Wang, A., Cao, G., Xu, J., Gao, L., 2009. Bioconversion of lignocellulosic biomass to hydrogen: Potential and challenges. Biotechnol. Adv. 27, 1051-1060.

Rodríguez-Abalde, A., Fernández, B., Silvestre, G.; Flotats, X., 2011. Effects of thermal pre-treatments on solid slaughterhouse waste methane potential. Waste Manage. 31, 1488-1493.

Rogalinski, T., Liu, K., Albrecht, T., Brunner, G., 2008. Hydrolysis kinetics of biopolymers in subcritical water. J. Supercrit. Fluid. 46, 335-341.

Ruiz, H.A., Rodríguez-Jasso, R.M., Fernandes, B.D., Vicente, A.A., Teixeira, J.A., 2013. Hydrothermal processing, as an alternative for upgrading agriculture residues and marine biomass according to the biorefinery concept: A review. Renew. Sust. Energ. Rev., 21, 35-51.

Ruiz, H., Ruzene, D.S., Silva, D.P., Quintas, M.A.C., Vicente, A.A., Teixeira, J.A., 2011. Evaluation of a hydrothermal process for pretreatment of wheat straw - effect of particle size and process conditions. J. Chem. Technol. Biot. 86, 88-94.

Saha, B.C., Yoshida, T., Cotta, M.A., Sonomoto, K., 2013. Hydrothermal pretreatment and enzymatic saccharification of corn stover for efficient ethanol production. Ind. Crop. Prod. 44, 367-372.

Sakaki T., Shibata M., Sumi T., Yasuda S., 2002. Saccharification of cellulose using a hot-compressed water-flow reactor. Ind. Eng. Chem. Res. 41, 661-665.

Sakdaronnarong, C., Srimarut, N., Lucknakhul, N., Na-songkla, N., Jonglertjunya, W., 2014. Two-step acid and alkaline ethanolysis/alkaline peroxide fractionation of sugarcane bagasse and rice straw for production of polylactic acid precursor. BioChem. Eng. J. 85, 49-62.

Saito, T., Sasaki, M., Kawanabe, H., Yoshino, Y., Goto, M., 2009. Subcritical Water Reaction Behavior of D-Glucose as a Model Compound for Biomass Using Two Different Continuous-Flow Reactor Configurations. Chem. Eng. Technol. 32, 527-533.

Sevilla, M., Fuertes, A.B., 2009. Chemical and structural properties of carbonaceous products obtained by hydrothermal carbonization of saccharides. Chem. Eur. J. 15, 4195-4203.

Scheller, H.V., Ulvskov, P., 2010. Hemicelluloses. Annu. Rev. Plant Biol. 61, 263-289.

Schumacher, B, Wedwitschka, H., Hofmann, J., Denysenko, V., Lorenz, H., Liebetrau, J., 2014. Disintegration in the biogas sector - Technologies and effects. Bioresource Technol. 168, 2-6.

Shen, S., Nges, I.A., Yun, J., Liu, J., 2014. Pre-treatments for enhanced biochemical methane potential of bamboo waste. Chem. Eng. J. 240, 253-259.

Srokol, Z., Bouche, A.G., Estrik, A.V., Strik, R.C.J., Maschmeyer T., Peters J.A., 2004. Hydrothermal upgrading of biomass to biofuel: studies on some monosaccharide model compounds. Carbohyd. Res. 339, 1717-1726.

Sun, P., Heng, M., Sun, S., Chen, J., 2010. Direct liquefaction of paulownia in hot compressed water: influence of catalysts. Energy 35, 5421-5429.

Sun, Y., Cheng, J., 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresource Technol. 83, 1-11.

Suryawati, L., Wilkins, M.R., Bellmer, D.D., Huhnke, R.L., Maness, N.O., Banat, I.M., 2009. Effect of hydrothermolysis process conditions on pretreated switchgrass composition and ethanol yield by SSF with Kluyveromyces marxianusIMB4. Process Biochem. 44, 540-545.

Taherzadeh, M.J., Karimi, K., 2008. Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review. Int. J. Mol. Sci. 9, 1621-1651.

Teghammar, A., Yngvesson, J., Lundin, M., Taherzadeh, M.J., Horváth, I.S., 2010. Pretreatment of paper tube residuals for improved biogas production. Bioresource Technol. 101, 1206-1212.

Triolo, J.M., Sommer, S.G., Møller, H.B., Weisbjerg, M.R., Jiang, X.Y., 2011. A new algorithm to characterize biodegradability of biomass during anaerobic digestion: Influence of lignin concentration on methane production potential. Bioresource Technol. 102, 9395-9402.

UNEP, 2009. Converting waste agricultural biomass into energy source.

Ververis, C., Georghiou, K., Danielidis, D., Hatzinikolaou, D.G., Santas, P., Santas, R., Corleti, V., 2007. Cellulose, hemicellulose, lignin and ash content of some organic materials and their suitability for use as paper pulp supplements. Bioresource Technol. 98, 296-301.

Vidal Jr., B.C., Dien, B.S., Ting, K.C., Singh, V., 2011. Influence of Feedstock Particle Size on Lignocellulose Conversion-A Review. Appl. Biochem. Biotech. 164, 1405-1421.

Wang, W., Yuan, T., Cui, B., Dai, Y., 2013. Investigating lignin and hemicellulose in white rot fungus-pretreated wood that affect enzymatic hydrolysis. Bioresource Technol. 134, 381-385.

Weil, J.R., Sarikaya, A., Rau, S.-L., Goebz, J., Lasisch, C.M., Brewer, M., Hendrickson, R., Tadisch, M.R, 1998a. Pretreatment of corn fiber by pressure cooking in water. Appl. Biochem. Biotech., 73, 1-17.

Weil, S., Brewer, M., Hendrickson, R., Sarikaya, A., Ladish, M.R., 1998b. Continuous pH monitoring during pretreatment of yellow poplar wood sawdust by pressure cooking in water. Appl. Biochem. Biotech. 70-72, 99-111.

Wilson, C.A., Novak, J.T., 2009. Hydrolysis of macromolecular components of primary and secondary wastewater sludge by thermal hydrolytic pretreatment. Water Res. 43, 4489-4498.

Wu, X., McLaren, J., Madl, R., Wang, D., 2010. Biofuels from lignocellulosic biomass. in: Singh, O.V., Harvey, S.P. (Eds.), Sustainable Biotechnology, Springer Press, Bradford, pp. 19-41.

Wyman, C.E. Ethanol production from lignocellulosic biomass: overview, In: Wyman, C.E. (Ed.), Handbook on Bioethanol, Production and Utilization. Taylor & Francis Press, Washington, DC, pp. 1-18.

Xie, S., Frost, J.P., Lawlor, P.G., Wu, G., Zhan, X., 2011. Effects of thermo-chemical pre-treatment of grass silage on methane production by anaerobic digestion. Bioresource Technol. 102, 8748-8755.

Yang, G., Pidko, E.A., Hensen, E.J.M., 2012. Mechanism of Brønsted acid-catalyzed conversion of carbohydrates. J. Catal. 295, 122-132.

Yu, G., Yano, S., Inoue, H., Inoue, S., Endo, T., Sawayama, S., 2010a. Pretreatment of rice straw by a hot-compressed water process for enzymatic hydrolysis. Appl. Biochem. Biotech. 160, 539-551.

Yu, Q., Zhuang, X.S., Yuan, Z.H., Wang, Q., Qi, W., Wang, W., Zhang, Y., Xu, J.L., Xu, H.J., 2010b. Two-step liquid hot water pretreatment of Eucalyptus grandisto enhance sugar recovery and enzymatic digestibility of cellulose. Bioresource Technol. 101, 4895-4899.

Zeng, M., Mosier, N. S., Huang, C. P., Sherman, D. M., Ladisch, M. R., 2007. Microscopic examination of changes of plant cell structure in corn stover due to hot water pretreatment and enzymatic hydrolysis. Biotechnol. Bioeng. 97, 265-278.

Zhang, Q., Tang, L., Zhang, J., Mao, Z., Jiang, L., 2011. Optimization of thermal-dilute sulfuric acid pretreatment for enhancement of methane production from cassava residues. Bioresource Technol. 102, 3958-3965.

Zhang, Z., Li, W., Zhang, G., Xu, G., 2014. Impact of pretreatment on solid state anaerobic digestion of yard waste for biogas production. World J. Microb. Biot. 30, 547-554.

Zhao, Y., Lu, W., Chen, J., Zhang, X., Wang, H., 2014. Research progress on hydrothermal dissolution and hydrolysis of lignocellulose and lignocellulosic waste. Front. Env. Sci. Eng. 8, 151-161.

Zheng, Y., Zhao, J., Xu, F., Li, Y., 2014. Pretreatment of lignocellulosic biomass for enhanced biogas production. Prog. Energy Combust. Sci. 42, 35-53.

Ziemiński, K., Romanowska, I., Kowalska-Wentel, M., Cyran, M., 2014. Effects of hydrothermal pretreatment of sugar beet pulp for methane production. Bioresource Technol. 166, 187-193.

Acknowledgements

Firstly, I deeply appreciate my supervisor, Professor Zhenya Zhang. During the three years of my doctoral program, thanks for his guidance, support, encouragement and friendship, I completed my studies in Tsukuba, Japan. Professor Zhang is always an excellent role model in my studies, his rigorous and conscientious attitude on research and affable manner are worth learning, I have a great esteem for him. And I would like to express my sincere appreciation to Associate Professor Zhongfang Lei, with her help I learned how to improve myself. Attribute to her many advices and encouragements, I could completed my studies smoothly. In addition, I want to express my great thanks to Associate Professor Motoo Utsumi, Associate Professor Yabar Mostacero Helmut Friedrich and Professor Yuhei Inamori. Owing to their patient reading, numerous beneficial suggestions and comments, the thesis could be accomplished promptly.

Moreover, I was also grateful to my seniors, school fellows and friends. Regardless of research and life, they gave me a large amount of research advices, learning communications and life assistances. More importantly, I would like to deliver my gratitude to my parents, this thesis would not have been accomplished without their love and support. The study experience of Doctoral Program in Sustainable Environmental Studies of University of Tsukuba was one of the most precious treasures in my life.