Enhancement of Methane Production from Solid-state Anaerobic Digestion of Yard Trimmings by Biological Pretreatment

THESIS

Presented in Partial Fulfillment of the Requirements for the Degree Master of Science in the Graduate School of The Ohio State University

By

Jia Zhao, B.S.

Graduate Program in Food, Agricultural and Biological Engineering

The Ohio State University

2013

Master's Examination Committee:

Dr. Yebo Li, Advisor

Dr. Jay F. Martin

Dr. Frederick C. Michel
Abstract

Anaerobic digestion (AD) is a biological process in which organic matter is decomposed by an assortment of microbes under oxygen-free conditions to produce biogas (about 40-70% CH$_4$ and 30-60% CO$_2$). AD is an efficient and mature waste treatment technology that can not only achieve the goal of waste disposal, but also generates biomethane, a renewable energy source. Based on its total solids percent (TS), AD can be classified as liquid AD (L-AD) or solid-state AD (SS-AD). Even though L-AD has a faster reaction rate and shorter retention time, SS-AD is generally thought to be advantageous over L-AD due to the smaller volume reactor equipment required, less energy input for heating, and because the solid byproduct weighs less, is more conductive to transportation and storage as compared to typical liquid AD effluents.

Yard trimmings are one of the main components of municipal solid waste. They are traditionally recycled by composting or disposed of by landfilling. In both process, energy is lost as heat. SS-AD could provide an alternative solution for the treatment of yard trimmings and the recovery of energy as biogas. The challenge of utilizing lignocellulosic biomass such as yard trimmings for SS-AD, however, is its recalcitrant structure, which impedes the hydrolysis of cellulose and hemicellulose. Lignin is believed to be one of the major factors inhibiting lignocellulose hydrolysis. Therefore,
pretreatment aimed at removing lignin from yard trimmings prior to SS-AD may improve the degradability of this feedstock.

In this study, two biological pretreatment methods for the enhancement of methane production during SS-AD were evaluated. These included fungal pretreatment by *Ceriporiopsis subvermispora* and composting. The fungal pretreatment study focused on the effect of moisture content (45%, 60% and 75%) and aeration mode (natural aeration, mechanical aeration for 15 min every 12 h and mechanical aeration for 30 min every 24 h) on the holocellulose and lignin loss and on methane yield during SS-AD. It was found that both aeration mode and moisture content had significant effects on the loss of hemicellulose and lignin. The highest lignin loss (20.9%) was obtained from naturally aerated yard trimmings with 60% moisture content as was the highest methane yield (44.6 L/kg VS(original yard trimmings)). Methane yield was positively linearly correlated with lignin degradation. Compared with the methane yield from original yard trimmings (without fungal pretreatment), fungal pretreatment increased the methane yield by up to 154%.

In addition, a simulated composting was used to pretreat a mixture of yard trimmings and AD effluent. The composting pretreatment was conducted at 45 °C with moisture content of 55% under natural aeration (no extra mechanical aeration) for 30 days. Composted yard trimmings and additional AD effluent were mixed and then fed into SS-AD reactors and placed at 37 °C for 30 days. The results showed no remarkable lignin loss during the composting process. The methane yield from the composted yard trimmings was not improved compared with the methane yield from original
yard trimmings (without composting pretreatment). Therefore composting pretreatment did not appear to be a promising approach to improvement of methane yield of yard trimmings during SS-AD.
This document is dedicated to my family.
Acknowledgments

I would like to express my appreciation to my advisor, Dr. Yebo Li for the support, guidance, and patience that he has provides throughout my graduate study. I would also like to thank my committee members: Dr. Jay F. Martin and Dr. Frederick C. Michel for their time to be my examination committee.

I would also like to express my gratitude to the staff members of Food, Agricultural and Biological engineering Department: Mike Klingmans for his dedication in providing the engineering assistance, feedstocks for my experiment, Mary Wicks and Peggy Christman for her enthusiasm in supporting my paper work. Of course, I would send my special thanks to Candy Mcbride for her response to all the administrative supports I needed, and her effort to make this department a home full of love. I really enjoy the time spent in the department with you all.

I am also indebted to my fellow lab members: Stephen Park, Dr. xumengGe, Dr. XiaolanLuo, Dr. Cong Li, Dr. Zhongjiang Wang, Dr. Jiying Zhu, Xinjie Tong for all of their help over the past year. I would give my specially thanks to Shengjun Hu and John Sheets, for the delighted time spent together with you guys on the basketball court. Surely, I cannot forget my roommate, Dr. Yi Zheng. I want to say thanks to him for his effort and time for my thesis revision, and also for the delicious meals.
I would also like to express my appreciation to Lo NieeLiew, Jessica Clutter from Quasar Energy Group, for their help during the past year.

Lastly, I would like to express my deepest gratitude to my family members for their constant understanding, patience and encouragement in no matter what I pursue.
Vita

October, 1989........................................Born in Shangqiu, Henan, China

July, 2011..............................................B.S. Biological Sciences, China
    Agricultural
    University, China

September, 2011 to present......................Graduate Fellow, Department of Food,
    Agricultural and Biological Engineering,
    The Ohio State University

Fields of Study

Major Field: Food, Agricultural, and Biological Engineering
Table of Contents

Abstract .................................................................................................................. ii
Acknowledgments .................................................................................................. vi
Vita .......................................................................................................................... viii
List of Tables .......................................................................................................... xii
List of Figures ......................................................................................................... xiii
Chapter 1. Introduction .......................................................................................... 1
Chapter 2. Literature Review ................................................................................... 5
  2.1. Anaerobic Digestion of Organic Wastes for Biogas Production ................... 5
    2.1.1. Fundamentals of anaerobic digestion process ...................................... 5
    2.1.2. Solid-state AD vs. liquid-AD ............................................................... 9
    2.1.3. Application of solid-state anaerobic digestion on various feedstocks ....... 10
  2.2 Pretreatment of Lignocellulosic Biomass for Biogas Production ................. 16
    2.2.1. Structural features of lignocellulosic biomass ..................................... 16
    2.2.2. Lignocellulosic biomass pretreatment ............................................... 17
    2.2.3. Biological pretreatment of lignocellulosic biomass for biogas production 19
  2.3. Composting used as a biological pretreatment method ............................... 26
2.3.1 Composting

2.3.2. Factors affecting composting

2.3.3. Biodegradation of lignin in composting environment

2.3.4. Composting pretreatment prior AD for enhancement of biogas production

Chapter 3. Enhancement of Methane Yield from Solid-state Anaerobic Digestion of Yard Trimmings by Fungal Pretreatment

3.1. Introduction

3.2. Materials and Methods

3.2.1. Feedstock and fungus preparation

3.2.2. Fungal pretreatment

3.2.3. Solid-state anaerobic digestion

3.2.4. Analytical methods

3.2.5. Data analysis

3.3. Results and Discussion

3.3.1. Degradation of yard trimmings by fungal pretreatment

3.3.2. Biogas production in SS-AD of pretreated yard trimmings

3.3.3. Loss of holocellulose during SS-AD

3.4. Conclusions

Chapter 4. Enhancement of Methane Yield from Solid-state Anaerobic Digestion of Yard Trimmings by Composting
4.1 Introduction ............................................................................................................................... 60
4.2 Materials and Methods ............................................................................................................ 63
  4.2.1 Materials ............................................................................................................................... 63
  4.2.2 Composting of the mixture of yard trimmings and liquid AD effluent ..................... 64
  4.2.3 Solid-state anaerobic digestion of composted yard trimmings ................................. 64
  4.2.4 Analytical methods ............................................................................................................. 65
  4.2.5 Statistical analysis ............................................................................................................. 66
4.3 Results and Discussion ............................................................................................................. 66
  4.3.1 Degradation of yard trimmings during composting ......................................................... 66
  4.3.2 Biogas production in SS-AD of composted yard trimmings ............................................ 68
  4.3.3 Loss of holocellulose during SS-AD of composted yard trimmings ......................... 72
4.4 Conclusions .............................................................................................................................. 73
Chapter 5. Conclusions and Suggestions for Future Research ..................................................... 74
Reference .......................................................................................................................................... 76
List of Tables

Table 2.1. Feedstock used in SS-AD for biogas production.............................11
Table 2.2. Biological pretreatment of lignocellulosic biomass for biogas production.20
Table 3.1. Chemical composition of yard trimmings ................................................35
Table 3.2. Experimental design of fungal pretreatment ...........................................37
Table 3.3. Two-way ANOVA on the effect of moisture content and aeration mode on lignin degradation during fungal pretreatment .......................................................47
Table 3.4. Tukey multiple comparisons of cumulative methane yield from yard trimmings pretreated under different conditions.................................................50
Table 3.5. Two-way ANOVA on the effect of moisture content and aeration mode on methane yield.........................................................................................50
Table 4.1. Degradation of holocellulose and hemicellulose during SS-AD............73
List of Figures

Figure 2.1. Main metabolic pathways during AD process (Li et al., 2011b).................. 6

Figure 2.2. Competition for acetate and hydrogen between sulfate-reducing bacteria and methanogens (Gerardi, 2003)............................................................................. 9

Figure 2.3. Mechanism of lignin degradation by ligninolytic enzymes (Chen et al., 2010) ........................................................................................................................................... 23

Figure 3.1. Effect of moisture content and aeration mode on the degradation of yard trimmings during fungal pretreatment: (a) dry mass loss; (b) cellulose degradation; (c) hemicellulose degradation; and (d) lignin degradation.................................................................................. 43

Figure 3.2. Accumulative methane yield from 40-day SS-AD of yard trimmings pretreated under different moisture contents and aeration modes (Data for the autoclaved and raw materials are not shown).................................................................................. 49

Figure 3.3. Linear correlation between lignin degradation and accumulative methane yield......................................................................................................................................... 52

Figure 3.4. Effect of moisture content and aeration mode in fungal pretreatment on methane content during 40-day SS-AD. (a) Natural aeration; b) 15 min/12 h; c) 30 min/24 h; d) comparison between fungal pretreatment, autoclave treatment and raw material on methane cont........................................................................................................ 53

Figure 3.5. Degradation of cellulose and hemicellulose during 40-day SS-AD (NA_60% means pretreatment under 60% moisture content and natural aeration)............................... 57

Figure 4.1. Process for composting and SS-AD ..................................................................................................................... 65
Figure 4.2. Degradation of yard trimmings during composting .......................... 67

Figure 4.3. Accumulative methane yield in 30-day SS-AD of composted yard trimmings (the Control group using original yard trimmings as feedstock) .................. 70

Figure 4.4. Methane yield at every determination .................................................. 71

Figure 4.5. Methane content during 30-day SS-AD .................................................. 72
Chapter 1. Introduction

Anaerobic digestion (AD) is a biological process in which organic matter is decomposed by an assortment of microbes under oxygen-free conditions and produce biogas (about 40-70% CH\textsubscript{4} and 30-60% CO\textsubscript{2}) (Frigon and Guiot, 2010). It is an efficient and widely used waste treatment technology with a by-product, digestate rich in nitrogen (Chen et al., 2008; Kenneher et al., 2002; Li et al., 2011b). As an efficient and mature waste treatment technology, AD has been widely used in the treatment of organic fraction of municipal solid waste (OFMSW), agricultural and industrial organic wastes (Chen et al., 2008). AD can not only achieve the goal of waste disposal, but also generates biomethane, a renewable energy source. It is reported (Braber, 1995) that a net energy production of 100-150 kWh per ton of municipal solid waste (MSW) could be achieved through AD. While for other waste disposal methods, like landfilling, incineration and composting, concerns of possible secondary pollution to the air and (or) ground water arise. (Mata-Alvarez et al., 2000)

Based on its total solids percent (TS), AD process can be categorized as either liquid AD (L-AD) or solid-state AD (SS-AD). In general, TS content of L-AD lies in the range of 0.5-15%, while SS-AD is usually operated with a TS content of 15% or greater (Rapport et al., 2008). Comparisons between L-AD and SS-AD indicate that L-AD has higher reaction rate and shorter retention time while SS-AD is believed to be advantageous over L-AD for a number of reasons, including smaller volume reactor required, less energy input for heating, minimal material handling, and less
total parasitic energy loss (Guendouz et al., 2010). Problematic issues related to floating and stratification of fibrous material in L-AD does not occur in SS-AD (Chanakya et al., 2009; Kaparaju et al., 2008). In comparison to the effluent of L-AD, the digestate of SS-AD is much easier to handle because of its lower water content (Li et al., 2011b). Animal manure, sewage sludge, and food waste are generally treated by L-AD, while organic fractions of municipal solid waste (OFMSW) and lignocellulosic biomass such as crop residues and food processing wastes can be processed through SS-AD. Using both SS-AD and L-AD to convert lignocellulosic feedstocks into biogas, Brown et al. (2012) revealed that the volumetric methane productivity was 2- to 7-fold greater in the SS-AD system in contrast to the L-AD system.

Feedstocks for AD are difficult to collect and transport (Frigon and Guiot, 2010). Compared to low total solid content feedstock, i.e., activated sludge, lignocellulosic biomass can be harvested at a high TS content, resulting in lower transportation costs per unit of solid. Yard trimmings (waste), which is composed of grass, leaves, and branches, is a major lignocellulosic source generated from households, municipalities, and landscaping companies and widespread through much of the US. The common technical route for yard trimmings treatment is composting, but the stored energy lost in the form of respiration heat (Koch et al., 2010). The utilization of yard trimmings by means of SS-AD can achieve waste disposal and the production of renewable energy, through a net energy production process.

Yard trimmings could be a suitable feedstock for SS-AD considering its easy availability and richness in carbohydrates. The challenges of utilizing these kinds of lignocellulosic biomass for AD are the recalcitrant properties which reduce their
biodegradability, making the hydrolysis step one of the bottlenecks that limit the production of methane (Zheng et al., 2009). Cellulose hydrolysis is slowed by the presence of lignin and hemicellulose, the high crystallinity and high degree of polymerization of cellulose, and the acetylation of hemicellulose. These inhibit hydrolytic enzymes from penetrating into the cellulose and hemicellulose (Tong et al. 1990; Zheng et al., 2009). Pretreatment is therefore necessary to improve the amenity of lignocellulosic biomass to bioconversion.

Due to the complexity and variability of biomass chemical structure and composition, the optimal pretreatment method and conditions greatly depends on the variety of the lignocelluloses feedstock. Several structural and compositional features have been found to impact on the biodegradability of lignocellulosic biomass, including cellulose crystallinity, accessible surface area, degree of cellulose polymerization, presence of lignin and hemicellulose, and degree of hemicellulose acetylation (Hsu, 1996; Kim and Holtzapple, 2005, 2006). Although many factors affect digestion, high lignin content is regarded as one of the most problematic factors that affects the bioconversion of lignocellulosic biomass (Liew, 2011). Yard trimmings contains an extremely high lignin content up to 38% (dry basis) making it resistant to AD. In order to increase the viability of yard trimming as a feedstock for AD, lignin removal by chemical pretreatment (e.g., NaOH) for improving biogas yield was conducted by Cherosky (2012) and Liew (2011). Depending on the dosage of NaOH pretreatment had negative, positive or no effect on the improvement of biogas yield of yard trimmings.
Compared with chemical pretreatment, biological pretreatment usually requires far less energy input. The processes are conducted under very mild environmental conditions and no inhibitors are generated for the following anaerobic digestion step. Although biological pretreatment requires long pretreatment time ranging from several days to several months, it could be overcome if the treatment can be done during biomass storage. Biological pretreatment for enhancement of biogas production in AD includes fungal pretreatment, pretreatment by microbial consortium and enzymatic pretreatment. Among these, fungus are the most common and effective reagents for delignification. In addition, significant lignin degradation also occurs in composting of lignocellulosic biomass (Tuomela et al., 2003). Well-controlled composting (i.e., controlled thermophilic phase for delignification) could be another pretreatment method for lignin removal from yard trimmings. Therefore, this thesis is focused on the fungal pretreatment and composting of yard trimmings.
Chapter 2. Literature Review

2.1. Anaerobic Digestion of Organic Wastes for Biogas Production

Anaerobic digestion (AD) is a biological process in which organic matter is decomposed by an assortment of microbes under oxygen-free conditions and produce biogas (about 40-70% CH$_4$ and 30-60% CO$_2$) (Frigon and Guiot, 2010). It is an efficient and widely used waste treatment technology with a by-product, digestate rich in nitrogen (Chen et al., 2008; Kenneher et al., 2002; Li et al., 2011b). AD is an efficient and mature waste treatment technology that has been widely used for the treatment of the organic fraction of municipal solid waste (OFMSW), agricultural and industrial organic wastes (Chen et al., 2008). AD can not only achieve the goal of waste disposal, but also produces renewable energy in the form of methane. It is reported (Braber, 1995) that a net energy production of 100-150 kWh per ton of municipal solid waste (MSW) could be achieved through AD.

2.1.1. Fundamentals of anaerobic digestion process

AD is a synergistic process carried out by coordinated actions of various groups of microbes. This process consists of a series of metabolic pathways, which can be mainly classified into four steps: hydrolysis, fermentation, acetogenesis, and methanogenesis (Figure 2.1) (Gerardi, 2003; Li et al., 2011b; Montero et al., 2008).
Hydrolysis

During hydrolysis process, insoluble complex organic wastes are degraded into simplistic soluble compounds, with the help of exoenzymes released by bacteria in AD systems. Exoenzymes are enzymes produced in cells and released outside of cells through cell membranes. Only part of bacteria have the ability of producing exoenzymes and usually one kind of bacterium can only produce exoenzymes degrading a specific substrate or group of substrates. For example, lipases can degrade lipids into fatty acids. Therefore, a diverse microbial community is needed in order to degrade various feedstocks present in AD reactors. Feedstocks of AD contain large amount of particulate and colloidal wastes, and thus hydrolysis step is necessary to
make them soluble to facilitate the following fermentation step. These feedstocks require relatively long digestion periods and thus for these feedstocks, hydrolysis is regarded as a rate-limiting step during AD process (Gerardi, 2003; Li et al., 2011b). For example, when lignocellulosic biomass is used as feedstock for AD, cellulases produced by cellulolytic bacteria enhance the hydrolysis of cellulose into simple sugars.

Fermentation

In fermentation stage, soluble substrates from hydrolysis and/or original feedstocks are converted by fermentative bacteria into volatile fatty acids (VFAs), alcohols, and simplistic inorganic compounds, such as carbon dioxide and hydrogen. Acetate acid, carbon dioxide and hydrogen produced in this step along with products derived from acetogenesis will be used as substrates of methanogens to produce methane (Li et al., 2011b).

Acetogenesis

During acetogenesis stage, VFAs and alcohols from fermentation stage are converted by acetogenic bacteria (or acetogens) to acetic acid or acetate, carbon dioxide, and hydrogen (Li et al., 2011b). Acetogens are sensitive to their metabolic waste (hydrogen), and can only survive under low hydrogen partial pressure. To keep the hydrogen pressure within the limitation, acetogens grow in a symbiotic relation with hydrogen-consuming bacteria (Gerardi, 2003). Since the generation of acetogenesis is a time-consuming process, which usually takes over 3 days, it is important to keep balanced microbial communities between acetogens and hydrogen-consuming bacteria (Pauss et al., 1990).
Methanogenesis

Methanogenesis is the final step of AD, where methane is produced from acetate, CO$_2$/H$_2$ by methanogens. Methanogens are oxygen-sensitive, fastidious anaerobes, belonging to the domain Archaebacetria. They are the only group that can produce methane (Gerardi, 2003). Based on the substrates used for methane production, methanogens are divided into acetoclastic methanogens and hydrogenotrophic methanogens. For acetoclastic methanogens, acetate is the substrate for the production of methane with CO$_2$ as a by-product. Acetoclastic methanogens play a dominant role in methane production, with around 70% methane from acetate. While for hydrogenotrophic methanogens, CO$_2$/H$_2$ are used for the production of methane. Together with sulfate-reducing bacteria, hydrogenotrophic methanogens are hydrogen-consuming bacteria that keep hydrogen in low concentration so that acetogens could perform their function of producing acids, CO$_2$ and H$_2$. Sulfate-reducing bacteria compete for substrate (acetates and H$_2$) with methanogens for the reduction of SO$_4^{2-}$ to H$_2$S and reproduction of sulfate-reducing bacteria (Figure 2.2). The produced hydrogen sulfide is inhibitory to acetogens and methanogens, and extra efforts might need to remove hydrogen sulfide from biogas depending on the application of biogas. Because of the long reproductive time and high sensitivity to oxygen, methanogens need a long retention time in anaerobic digesters to produce a large population. Thus, methanogenesis is regarded as a rate-limiting step during AD process (Gerardi, 2003; Montero et al., 2008; Zinder, 1993).
2.1.2. Solid-state AD vs. liquid-AD

Based on the total solid (TS) content, AD can be classified into liquid-AD (L-AD) (TS<15%) and solid-state AD (SS-AD) with TS over 15% (Rapport et al., 2008). Compared with L-AD, SS-AD requires less reactor volume, lower energy requirement for heating and stirring, minimal material handling, and lower total parasitic energy loss (Guendouz et al., 2010). Problems related to the floating and stratification of fibrous material in L-AD could be solved with SS-AD (Chanakya et al., 2009; Kaparaju et al., 2008). Digested residue from SS-AD is more concentrated and easier to be disposed and more suitable for fertilizer than that from L-AD. Despite these advantages of SS-AD over L-AD, SS-AD usually has lower reaction rate so that the retention time of SS-AD can be up to three times longer than L-AD. Hence, it is a case by case situation to decide whether SS-AD or L-AD is more economic (Brummeler et al., 1991; Schäfer et al., 2006; Singh and Anand, 1994).
2.1.3. Application of solid-state anaerobic digestion on various feedstocks

SS-AD has been widely used in the disposal of organic wastes with high solid content, such as animal wastes, food waste, agricultural wastes, municipal solid wastes, energy crops and sometimes even dewatered waste water sludge (Jha et al., 2011; Li et al., 2011b). Feedstocks having been used in SS-AD are shown in Table 2.1.
Table 2.1. Feedstock used in SS-AD for biogas production

<table>
<thead>
<tr>
<th>Feedstocks</th>
<th>Reactor type</th>
<th>Total solid</th>
<th>AD conditions</th>
<th>Results</th>
<th>Comments</th>
<th>Selected references</th>
</tr>
</thead>
<tbody>
<tr>
<td>OFMSW (including water-sorted OFMSW, SS-OFMSW, and MS-OFMSW), some of</td>
<td>Batch and fed-batch reactors</td>
<td>16-35%</td>
<td>30-60 °C for 25 days to 1 year,</td>
<td>Methane yield ranged from 130 L/kg VS to 400</td>
<td>Some reactors have lecachate recycle; SS-OFMSW has higher methane yield</td>
<td>Brummeler et al. (1991), Guendouz et al. (2010), Bolzonella et al. (2006), Kayhanian and Rich (1995), Chaudhary (2008), Li et al. (2010), Brummeler and Koster (1990), Fernández et al. (2008)</td>
</tr>
<tr>
<td>them co-digestion with sludge, dairy manure, and grey waste.</td>
<td></td>
<td></td>
<td>completely, discontinuously</td>
<td>L/kg VS. Some of them reached 70% of the</td>
<td>than MS-OFMSW;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>mixed or no mixing</td>
<td>potential methane yield</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animal manure (swine poultry, dairy manure, horse dung), most of them</td>
<td>Batch and fed-batch reactors</td>
<td>15-30%</td>
<td>30-60 °C for 40 days to 3 months</td>
<td>170 L/kg VS (methane) to 800 L (biogas)/kg VS</td>
<td>Animal manure is usually not as a solo feedstock for AD. Co-digestion</td>
<td>Ahn et al. (2010), Hall et al. (1985), Kayhanian and Rich (1995), Kusch et al. (2008), Li et al. (2011b)</td>
</tr>
<tr>
<td>co-digestion with straws, sludge, and OFMSW</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>with lignocellulosic biomass.</td>
<td></td>
</tr>
<tr>
<td>Agricultural wastes and energy crops (Corn stover, switchgrass, fall</td>
<td>Batch and fed-batch reactors</td>
<td>20-30%</td>
<td>30-55 °C for 30 days to 1 year</td>
<td>Methane yield ranged from 82 to 372 L/kg VS</td>
<td>Pretreatment or co-digestion with manure is necessary for good</td>
<td>Ahn et al. (2010), Bolzonella et al. (2006), Cui et al. (2011), Hall et al. (1985), Kayhanian and Rich (1995), Kusch et al. (2008), Liew et al. (2011), Zhu et al. (2010),</td>
</tr>
<tr>
<td>leaves, wheat straw, and grey waste)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>performance.</td>
<td></td>
</tr>
</tbody>
</table>

Continued
Table 2.1. Feedstock used in SS-AD for biogas production (Continued)

<table>
<thead>
<tr>
<th>Feedstocks</th>
<th>Reactor type</th>
<th>Total solid</th>
<th>AD conditions</th>
<th>Results</th>
<th>Comments</th>
<th>Selected references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sludge (waste water sludge, dewatered sludge)</td>
<td>Batch and fed-batch reactors</td>
<td>15-30%</td>
<td>35-60 °C for 63 days to 1 year</td>
<td>From 130 L( methane) /kg VS to 800 L(biogas)/kg VS</td>
<td>Sludge without being dewatered, is usually co-digested with other high solid wastes. Dewatered sludge was tried for the first time for SS-AD, with methane yield of 340 L/kg VS.</td>
<td>Bolzonella et al. (2006), Duan et al. (2012), Kayhanian and Rich (1995), Li et al. (2011a)</td>
</tr>
</tbody>
</table>


Organic Fraction of Municipal Solid Waste (OFMSW)

Among the solid organic wastes, MSW is one of the most commonly treated by SS-AD, considering its high solid content and large quantity worldwide. At present, around two billion tons of MSW is generated per year, which is still increasing (Khalid et al., 2011). The composition of MSW varies according to the region, climate, season, collection frequency, and many other factors (Gunaseelan, 1997). In 2011, US produced 250 million tons of MSW, among which paper and paperboard accounted for about 29%; food scraps and yard trimmings take up 27%; and metals, plastics, rubber, leather, textiles, and other non-biodegradable materials accounted for about 30% (EPA, 2011). The sorting method can significantly influence the quality of OFMSW for SS-AD for methane production. Usually, source-sorted OFMSW (SS-OFMSW) has higher biogas yield than mechanical-sorted OFMSW (MS-OFMSW). Bolzonella et al. (2006) reported that SS-OFMSW had methane yield of 400L/kg VS under mesophilic conditions, while MS-OFMSW combined with sludge and grey waste had methane yield as low as 130L/kg VS. Besides the difference of biogas production, the SS-AD digestates of SS-OFMSW and MS-OFMSW also have different fate: residues from SS-OFMSW could be composted or fertilizer, while residues from MS-OFMSW should be disposed in landfills or incineration (Bolzonella et al., 2006). Chaudhary (2008) revealed that methane yield of SS-OFMSW reached 278L/kg VS at 55°C. Li et al. (2010) reported that methane production from water-sorted OFMSW, which is largely available in China, was higher than that from MS-OFMSW and even comparable to that from SS-OFMSW. OFMSW is considered a
suitable feedstock for SS-AD, however, Kayhanian and Rich (1995) believed that typical OFMSW is insufficient in nutrients for microbial community’s normal function and found that the addition of wastewater sludge and dairy manure could increase biogas production and process stability.

Animal manure

Animal manure is mainly composed of easily degradable organic matters with high organic nitrogen. The hydrolysis is not the rate-limiting step for this feedstock during AD. Bujoczek et al. (2000) indicated that high solid content in reactors with animal manure as feedstock can cause ammonia inhibition and volatile fatty acid (VFA) accumulation, both of which might lead to the failure of reactors. They found that high solid AD with 10% TS using chicken manure as feedstock had the optimal biogas production, and TS over 10% deteriorated the performance of reactors in terms of biogas production. As a result, animal manure seems not suitable to be a solo-substrate for SS-AD in the conventional single-stage reactors. Co-digestion with other wastes of high C/N ratio to adjust the total C/N ratio makes it possible to achieve high solid content in AD reactors. Investigation has been conducted in the feasibility of co-digestion of manure with agricultural wastes, energy crop, MSW, and even wastewater sludge and better performance was achieved (Ahn et al., 2010; Cui et al., 2010; Hall et al., 1985; Kayhanian and Rich, 1995; Kusch et al., 2008; Li et al. 2011b). Two-phase operation, separating methanogenesis and the other three steps into two different reactors, provides another way for achieving high-solid content AD for
animal manure. Since methanogens are less tolerant to excess ammonia and more sensitive to low pH, compared with the other three classes of microbes in AD, separating methanogenesis and control the loading rate into methanogenesis reactor can remove the toxicity caused by excess free ammonia and low pH caused by VFA accumulation (Bujoczek et al. 2000; Chen et al., 2008; Li et al., 2011b, and Mata-Alvarez et al., 2000).

_Food waste_

Food waste is similar to animal manure in low total solid, high content of soluble organic content but it is much more easily degradable and has a higher energy content per dry mass. Excess ammonia and VFA accumulation are faced with AD of high solid content food waste. Co-digestion of food waste with lignocellulosic biomass and/or two-phase operation was used to solve aforementioned problems (Chen et al., 2008, Li et al., 2011a, and Mata-Alvarez et al., 2000).

_Agricultural wastes and energy crops_

Agricultural wastes and energy crops are mainly lignocellulosic biomass, composed of cellulose, hemicellulose and lignin. Structural and compositional features of lignocellulosic biomass make it highly recalcitrant to both chemical and biological hydrolysis. As a result, hydrolysis of such resistant feedstock is a rate-limiting step in AD. To better utilize the carbohydrates of lignocellulosic feedstock, pretreatment is usually necessary to break down its structures. Zhu et al., (2010) found that corn
stover pretreated by 5% NaOH at ambient temperature (20 ± 0.5°C) for 24 h had 37% increase in methane production reaching 372.4 L/kg VS. The pretreatment of lignocellulosic biomass will be detailed in the next section. Besides the rigid structure, the carbon to nitrogen (C/N) ratio of this kind of substrates is usually much higher than the theoretical optimal C/N ratio (25-30) for SS-AD because of the high content of carbohydrates and lignin. Therefore, co-digestion of lignocellulosic feedstock with other wastes (e.g. manure and/or food waste) of low C/N ratios is necessary to balance the nutrient in AD reactors. Animal manure is of high content of organic nitrogen, which results in the low C/N ratio of this kind of waste (Bujoczek et al., 2000 and Hansen et al., 1998). Zhang et al. (2007) investigated the characterization of food waste and found that the average C/N ratio was 14.8, which also made food waste a candidate for the co-digestion of agricultural wastes.

2.2 Pretreatment of Lignocellulosic Biomass for Biogas Production

2.2.1. Structural features of lignocellulosic biomass

Lignocellulosic biomass, as one the most abundant organic matters on the planet, mainly comprises a large fraction of municipal solid waste, agricultural and forest residues, and energy crops. The abundance, richness in carbohydrates (55-75% dry basis), and non-competition with food make lignocellulosic biomass a suitable feedstock for the production of biofuels such as biogas (Chen et al., 2010; Taherzadeh and Karimi, 2008; Takara and Khanal, 2012; Wan and Li, 2012; Zheng et al., 2009).
Lignocellulosic biomass is mainly composed of cellulose, hemicelluloses and lignin. Cellulose is a linear polymer of glucose linked by \( \beta 1-4 \) glycosidic bonds. The cellulose chains are stabilized by hydrogen bonds, and connected by hemicelluloses. Hemicellulose is a heteropolymer of many different 5-carbon and 6-carbon sugars. Both carbohydrates can provide fermentable sugars for the metabolism of microbes after hydrolysis. Cellulose and hemicelluloses is woven together by lignin, a cross-linked polymer of p-coumaryl alcohol, cooniferyl alcohol, and sinapyl alcohol. The presence of lignin further reinforce the structure of lignocellulosic biomass. Among three main components, lignin is the most recalcitrant polymer. In general, the higher lignin content, the more resistant to hydrolysis lignocellulosic biomass becomes.

Recalcitrant features of lignocellulosic biomass render it highly resistant to chemical and biological degradation. To increase the AD efficiency of lignocellulosic biomass for biogas production, pretreatment of feedstock is necessary to break down the structural obstacles and thus facilitate the hydrolysis step in AD process.

2.2.2. Lignocellulosic biomass pretreatment

Sharing the same step of hydrolysis of lignocellulosic biomass into fermentable sugars for the production of cellulosic bioethanol, Biogas production from AD faces the similar problems to ethanol production. Recalcitrance of lignocellulosic biomass hinders the hydrolysis of cellulose and hemicelluloses. Therefore, the pretreatment methods in bioethanol production are also applicable to biogas production.
The pretreatment methods can be classified into three categories: physical, chemical, and biological pretreatment methods. Physical pretreatment refers to the pretreatment process without addition of chemicals (water is not regarded as chemicals in this process). It mainly includes comminution (e.g. mechanical milling and grinding), irradiation (ultrasound and microwave), extrusion, and high pressure steaming. This pretreatment facilitates the hydrolysis of carbohydrates mainly by increasing the interaction surfaces between carbohydrates and enzymes and/or chemicals, and solubilization of hemicelluloses. It can also alter lignin structure and solubilize lignin. This method can generally improve methane yield of lignocellulosic biomass. However, considering the high energy consumed during this process, physical pretreatment is not applicable in commercial production (Zheng et al., 2009).

Chemical pretreatment utilizes chemicals, such as alkali, acid, ozone, etc. to remove lignin and/or hemicelluloses, decrease cellulose crystallinity, and increase interaction surfaces. It includes alkaline pretreatment, acid pretreatment, catalyzed steam-explosion, wet-oxidation, ozonolysis, oxidative pretreatment with peroxides, and ionic liquid pretreatment (Zheng et al., unpublished). Chemical pretreatment is effective and rapid, and considered the most promising for industrial application. However, harsh reaction conditions, corrosion of reactors, utilization of chemicals, and possible inhibitors for the following hydrolysis and methanogenes are barriers for further application (Taherzadeh and Karimi, 2008; Takara and Khanal, 2012). Compared with physical and chemical pretreatment, biological pretreatment requires no extra chemicals or energy inputs, mild reaction conditions, and low costs in reactors (Wan
and Li, 2010b; Taherzadeh and Karimi, 2008). It utilizes microbes and/or enzymes to mainly remove lignin and improve hydrolysis of carbohydrates polymer. The concern of long pretreatment time can be reduced and/or eliminate, since biological pretreatment could be easily integrated with on-farm wet storage (Wan and Li, 2010b). Previous research has shown biological pretreatment can effectively enhance biogas production from AD of various lignocellulosic biomass (Amirtaea al., 2006; Jasko et al., 2013). This work mainly focuses on biological pretreatment.

2.2.3. Biological pretreatment of lignocellulosic biomass for biogas production

Biological pretreatment for biogas enhancement in AD mainly includes fungal pretreatment, pretreatment by microbial consortia, and enzymatic pretreatment. Biological ensiling was occasionally used as a pretreatment technique to improve biogas yield of lignocellulosic biomass, but only a slight effect of ensiling was found by Vervaeren et al (2010). Therefore, ensiling is not further discussed in this review. Table 2.2 is the summary of biological pretreatment methods used in lignocellulosic biomass to increase biogas production.
### Table 2.2. Biological pretreatment of lignocellulosic biomass for biogas production

<table>
<thead>
<tr>
<th>Biological pretreatment</th>
<th>Microbes and enzymes</th>
<th>Feedstocks</th>
<th>Pretreatment conditions</th>
<th>Results</th>
<th>Selected references</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fungal pretreatment</strong></td>
<td>White-, brown-, and soft-rot fungi, e.g. <em>Ceriporiopsissubvermispora, Auriculariaauricula-judae, Trichodemareesii</em>, and basidiomycete fungi (e.g. <em>Ischnodermaresinosum, pleurotusostreatus</em> and <em>Fomitellafrauxinea</em>)</td>
<td>Agricultural residuals: sweet chestnut leaves/hay, deciduous tree sawdust and sisal leaf decortications residue</td>
<td>28-37°C for 12 days to 8 weeks</td>
<td>15% to 5 folds increase of methane yield</td>
<td>Amirta et al. (2006), Jasko et al. (2013), Mackulak et al. (2012), Muthangya et al. (2009), and Take et al. (2006)</td>
</tr>
<tr>
<td><strong>Microbial consortium</strong></td>
<td>Complex microbial agents containing yeast and cellulolytic bacteria, heat-treated sludge, <em>Clostridium thermocellum</em>, and mixture of fungi and composting microbes</td>
<td>Agricultural residuals: corn straw, corn stalks, cotton stalks, cassava residues, and biofiber of manure</td>
<td>20-55°C for 12 h-20 days</td>
<td>Methane yield improvement by 25-96.63%</td>
<td>Bai et al. (2010), Bruni et al. (2010), Lu et al. (2009), Zhang et al. (2011), and Zhong et al. (2011)</td>
</tr>
<tr>
<td><strong>Enzymatic pretreatment</strong></td>
<td>Laccase, pectinase, mixture of cellulase and hemicellulases, mixture of cellulase, hemicellulase and β-glucosidase, and crude <em>Trichoderma</em> enzyme complex</td>
<td>Agricultural residuals: Sugar beet pulp, spent hops and biofiber of manure</td>
<td>37 °C for 4-24 h</td>
<td>0-34% increase of methane yield</td>
<td>Bruni et al. (2010), Gerhardt et al. (2007), Lin et al. (2010), Romano et al. (2009), and Ziemiński et al. (2012)</td>
</tr>
</tbody>
</table>

20
<table>
<thead>
<tr>
<th>Biological pretreatment</th>
<th>Microbes and enzymes</th>
<th>Feedstocks</th>
<th>Pretreatment conditions</th>
<th>Results</th>
<th>Selected references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ensilaging</td>
<td>Mixture of homo- and hetero-fermentative bacteria, mixture of lactic acid bacteria and enzymes (α-amylase, cellulase, hemicellulase, and pentosanase), and mixture of homo- and hetero-fermentative lactic acid bacteria, yeast and fungi</td>
<td>• Agricultural residuals: maize</td>
<td>Room temperature for 7 weeks</td>
<td>15% increase of methane yield, but negative effect was also found</td>
<td>Vervaeren et al. (2010)</td>
</tr>
</tbody>
</table>
Fungal pretreatment

Research on fungal pretreatment mainly focused on fungi selectively degrading lignin and hemicellulose, while utilizing little cellulose. By degrading lignin and hemicellulose, the digestibility of cellulose is increased, which is preferred for the following anaerobic digestion. Fungal pretreatment is usually conducted in sterilized substrate without any other microorganisms except for the fungus. Several fungi classes, e.g. brown-, white- and soft-rot fungi, have been used for pretreatment of lignocellulosic biomass for biogas production. In general, white rot fungi is the most used and effective biological pretreatment method, and has been applied to biopulping in paper industry for a long time (Taherzadeh and Karimi, 2008; Wan and Li, 2012). What is more, there are many species of white rot fungi can selectively degrade lignin and (or) hemicelluloses over cellulose, which is preferred substrates for the following fermentation for the production of biofuels, like biogas (Narayanaswamy et al., 2013).

There are about 1500 species of white-rot fungi can selectively degrade lignin over cellulose (Narayanaswamy et al., 2013). White-rot fungi degrade lignin with the assist of ligninolytic enzyme system (Figure 2.3). Since lignin does not contain enzymatically hydrolysable linkages, the enzymes or agents degrading lignin must be oxidative. The major enzymes involved in the process are three phenol oxidases: Laccases, lignin peroxidases (Lip), and manganese peroxidases (MnP). However, not all the three enzymes can be secreted by all white-rot fungi. Laccases and MnP are more common than LiP (Chen et al., 2010; Wan and Li, 2012).
Because the integrated structure of lignocelluloses prevents the enzymatic and microbial attacks, radical reactions play an important role in the degradation of lignin. Ligninolytic enzymes have no direct binding with lignin units. It is with the assist of oxidized donors that ligninolytic enzymes achieved the degradation of lignin. These donors come from the oxidization of small molecules catalyzed by ligninolytic enzymes. The oxidized donors catalyze the removal of an electron from phenolic and aromatic amino groups forming phenolic radicals and aromatic radicals (Tian et al., 2011; Wan and Li, 2012). LiP uses H2O2 as a co-factor to oxidize non-phenolic units of lignin by removing one electron and creating free radicals. MnP, similar to LiP, needs H2O2 to convert Mn^{2+} to Mn^{3+}. Mn^{3+} then oxidizes phenolic rings to phenoxy radicals. Laccases can catalyze single-oxidations of phenolic compounds in lignin with the reduction of O2 to H2O. Besides the
aforementioned three enzymes, there are also versatile peroxidases (VP), and manganese-independent peroxidases (MiP) catalyzing lignin degradation process. VP has the ability of both LiP and MnP, and MiP can perform the function of MnP in the absence of manganese ions. Accessory enzymes can further enhance delignification by creating cofactors. For example, glyoxal oxidases (GLOX) catalyze the production of H$_2$O$_2$, which is an important co-factor for LiP and MnP (Chen et al., 2010; Narayanaswamy et al., 2013; Tian et al., 2011). Mackuľak et al. (2012) used Auricularia auricula-judae, a wood-decaying fungus to pretreat the mixture of sweet chestnut (Castanea sativa) leaves and hay at 37 °C for 4-5 weeks. The pretreated mixture (leaves/hay= 1:2) had a 15% increase in biogas production compared with unpretreated samples. Take et al (2006) compared methane production from Japanese cedar wood chips pretreated by different methods, and found that fungal pretreatment by Cyathus stercoreus AW 03-72 and Trametes hirsute NBRC 4917 had better effect than steaming pretreatment and was comparable to combined pretreatment of steaming and refiner. The pretreatment effect on Japanese cedar wood chips with four fungi was investigated by Amirta et al (2006) who indicated that wood chips pretreated by Ceriporiopsis subvermispora ATCC 90467 with the presence of wheat bran for 8 weeks had the best performance with 4 times higher methane yield than the control. Two-stage fungal pretreatment was also investigated and had positive effect on methane production (Muthangya 2009). CCTH-1 was inoculated in sisal leaf decortications residue (SLDR) for 4 days followed by the inoculation of Trichoderma reesei for another
8 days, or in the reversing order. Both of the pretreatment could increase methane yield, but with different enhancement for different inoculation orders.

**Microbial consortium pretreatment**

Microbial consortium is constructed by screening samples from wild environment from rotten lignocellulosic biomass. Different from fungal pretreatment mainly attacking lignin, microbial consortium is usually of high cellulose-degradation ability and hemicellulose-degradation ability. A thermophilic microbial consortium constructed by Zhang et al (2011) from soil samples from wild environment, such as decaying straw and thermophilic landfill. After pretreated by the consortium mixing with distillery wastewater at 55°C for 12 h, cassava residues had over 96% higher methane yield compared with the control. Using multigeneration selection method, Bai et al. (2010) screened a microbial system named MEG which was able to increase cotton stalks’ biogas production by 25%, after pretreating cotton stalks at 35±2 °C for 7 days. However, when the mixture of compost from garden waste and fungi from maize silage were applied to biofibers in manure, there was no improvement in biogas production. (Bruni et al 2010). Besides the consortium screened from the wild, the complex microbial agents in the form of freeze-dried powders containing yeast and cellulolytic bacteria were inoculated in autoclaved corn straw (Zhang et al., 2011). With corn straw pretreated for 15 days at ambient temperature, the methane yield increased by over 75% with a 34.6% reduction in digestion time. Sterilization of lignocellulosic substrates is usually not
necessary when using microbial consortium for pretreatment, except for the situation with a mixture of pure-strains.

**Enzymatic pretreatment**

In the anaerobic digestion of lignocellulosic biomass, hydrolysis of cellulose and hemicelluloses is the rate-limiting step (Romano et al., 2009). To increase the biogas production, enzymes with hydrolytic activities were applied before or in the anaerobic digestion. The most common used enzymes were cellulase and hemicellulase. Mushroom compost extract containing laccase and CMC (carboxymethylcellulose)ase activities was used to pretreat pulp and paper sludge (PPS) and resulted in over 34% increase of methane yield. This method was impressive because of the short pretreatment time (4 h) and low costs compared with other biological pretreatment methods (Lin et al, 2010). In most cases, the effect of enzymes in enhancing biogas production was insignificant, and the high cost of enzymes was another issue which should be taken into account.

**2.3. Composting used as a biological pretreatment method**

**2.3.1 Composting**

Composting is a process involving biological decomposition of organic through the synergistic actions of microorganisms under controlled aerobic conditions into CO$_2$, biomass, heat and a humus-like end product (Esptein 1997; Tuomela et al., 2000; Liang et al., 2003). The organics are mainly carbohydrates (e.g. starch, cellulose, and hemicelluloses), proteins, lipids, and lignin. Composting has been a mature method used
for the disposal of organic wastes, such as municipal solid waste, agricultural residues, wastewater sludge, and other industrial organic effluent. Easily degradable feedstock is consumed by microbes in composting environment, and the residuals are usually converted to humus-like products which can be used as fertilizer (Tuomela et al., 2000; Vargas-Garcia et al., 2007).

Typical composting process comprises four phases, namely mesophilic phase, thermophilic phase, cooling phase, and maturation phase. Different microbes dominate in different phases, and the length of every phase varies depending on feedstocks and operation conditions. In the mesophilic phase which is also the beginning of composting, microbes consume soluble and easily degradable organics, such as free sugars, starch, lipids, and proteins (Tuomela et al., 2000). During this phase, mesophilic bacteria dominate, even though there do exist mesophilic fungi. However, fungi are outcompeted by the rapid growth of bacteria, considering the high surface/volume ratio and shorter generation time of bacteria. Because of the heat produced from mesophilic microbes’ activity, the temperature of compost increases. After temperature reach 40°C, thermophilic microbes take over in the composting process, indicating the start of thermophilic phase. In this phase, actinomycetes start to strive. Together with thermophilic fungi, actinomycetes are responsible for the degradation of natural polymers. The temperature continues to increase because of the activity of thermophilic microbes. When temperature is over 60°C, all microbial activities decrease, leading to the drop in temperature, indicating the coming of cooling phase or the second mesophilic phase. In
this phase, mesophilic bacteria and mesophilic fungi reappear, and actinomycetes still exist. The number of microbes might be smaller than that in the thermophilic phase, however, the metabolic diversity increases. The degradation of natural complex polymers continues in the phase (Ryckeboer et al., 2003; Tuomela et al., 2000).

2.3.2. Factors affecting composting

There are several critical factors affecting composting, including C/N ratio, moisture content, temperature, and aeration. Both carbon and nitrogen are important nutrients, and the optimum C/N ratio is around 25-40 (Tuomela et al., 2000). Moisture also plays an important role, considering the fact that organic molecules have to dissolve in water prior to being used by microbes. Moisture cannot only influence the growth of fungi by the dissolved organics, but also has an effect on aeration. Liang et al. (2003) found that 50% moisture was the upper critical level for an effective composting. Excessive moisture, e.g. over 70%, would cause the lack of oxygen. Aeration is vital during composting process, for most of the microbes during this process are aerobics. Especially during the mesophilic phases, respiration provides the heat for reaching thermophilic phases. During thermophilic phases, there is also great oxygen-consumption observed (Tomati et al., 1995). As mentioned in last part, thermophilic phase played an important role in lignin degradation, especially the activity of thermophilic fungi, which have the highest ability of lignin degradation among fungi, bacteria, and actinomycetes. So keeping the optimal condition for the growth of thermophilic fungi is critical in lignin degradation. The optimal temperature for most thermophilic fungi ranges from 40°C to 50°C. (Tumela et
al., 2000) The conditions mentioned above provide the reference for a success composting. However, these values might vary depending on the substrates.

2.3.3. Biodegradation of lignin in composting environment

As mentioned in the biological pretreatment part, lignin degradation in nature mostly attributed to the activity of basidiomycotina, the majority of which are mesophiles. There is a small group of basidiomycotina and some ascomycotina growing well in thermophilic temperature. These thermophilic fungi could be found in garden compost heaps, bird nests, power plant cooling pipes and effluents, in the storage of agricultural products, and in piles of wood chips and peat. There were white-rot fungi, brown-rot fungi, soft-rot fungi found in the thermophilic phase. Most of them have great ligninolytic activity and the optimal temperature for most thermophilic fungi is between 40°C and 50°C, which is also the optimal temperature for the degradation of lignin. In the study of Galli et al. (1997) and Tomati et al. (1995), the lignin in the mixture of olive-mill wastewater-wheat straw was degraded by 70% at the end of thermophilic phase, while no further lignin degradation was detected during maturation phase. During maturation phase, only low molecular weight soluble lignin fractions released in thermophilic phase were humified.

Besides thermophilic fungi, actinomycetes appearing in thermophilic phase and cooling and maturation phases, are also able to solubilize lignin. However, the ability to degrade lignin is not as good as that of fungi. Huang et al. (2010) used quinone profiling as the
indicators of the existence of certain microbes during different phases of composting. They found that together with fungi, bacteria, actinomycetes played an important role in the degradation of lignin in the cooling phase. In their study, MK-7 was found to be correlated with lignin degradation in both thermophilic and cooling phases. MK-7 is considered an indicator of bacteria such as *Bacillus* spp.. This result is consistent with the research that typical bacteria of thermophilic phase are species of *Bacillus* (Tuomela et al., 2000).

2.3.4. Composting pretreatment prior AD for enhancement of biogas production

Besides as a way to dispose organic wastes getting humics-rich end products, composting has also been applied in the improvement of AD of OFMSW. Because of the high amount of easily degradable organics in OFMSW, acidification sometimes happened during the start-up of AD systems (Charles et al., 2009). To avoid the acidification, which might cause the accumulation of VFA and lead to the failure of AD reactions, composting prior AD could remove the easily degradable organics (Charles et al., 2009; Brummeler and Koster, 1990). However, the property of lignin degradation during composting has never been used to improve biogas production. This property can be a potential solution to the recalcitrance of lignocellulosic biomass hindering the hydrolysis of cellulose during AD process.

In summary, SS-AD was used to digest yard trimming for biogas production in this study due to its aforementioned advantages. In order to improve biogas yield of yard trimming
in SS-AD, biological pretreatment, i.e. fungal pretreatment and composting method was employed to make yard trimming more degradable.
Chapter 3. Enhancement of Methane Yield from Solid-state Anaerobic Digestion of Yard Trimmings by Fungal Pretreatment

Yard trimmings, as a main component of municipal solid waste (MSW), are potential feedstock for solid-state anaerobic digestion (SS-AD) to produce methane. Due to their structural and compositional recalcitrance to biodegradation, however, pretreatment is usually needed to make yard trimmings more degradable in AD. In this study, yard trimmings were pretreated by Ceriporiopsis subvermispora, a white rot fungus that selectively degrades lignin, to enhance methane yield of yard trimmings. The pretreatment study was focused on the effect of moisture content (with three levels of 45%, 60% and 75%) and aeration mode (natural aeration (without mechanical aeration), aeration for 15 min/12 h and aeration for 30 min/24 h) on the holocellulose and lignin loss and methane production of pretreated yard trimmings through SS-AD. It was found that C. subvermispora had a selective loss of lignin up to 20.9%, but little cellulose loss (around 7%). The yard trimmings pretreated under 60% moisture content and natural aeration had the highest methane yield of 44.6 L/kg VS (original yard trimmings) which was over 2 times higher than that of the autoclaved yard trimmings without fungal pretreatment (21.6 L/kg VS (original yard trimmings)) and almost three folds over that of the raw yard trimmings (17.6 L/kg VS (original yard trimmings)). The enhancement of
methane yield is probably caused by the lignin loss during fungal pretreatment. Methane yield increased linearly with the increase of lignin loss.

3.1. Introduction

Yard trimmings are one of the main components of municipal solid wastes. In 2011, there was about 250 million tons of municipal solid waste (MSW) generated in US, among which yard trimmings took 13.5% (EPA, 2011). Yard trimmings could be a suitable feedstock for solid-state anaerobic digestion (SS-AD) to produce methane, due to their abundance, carbohydrate components, and the potential for extra income from tipping fees associated with recycling of this kind of biomass. However, the methane yield from SS-AD of yard trimmings is lower than that of other lignocellulosic biomass, such as corn stover (Brown, 2012), mainly due to the structural and compositional recalcitrance to biodegradation. The complex structure of lignocellulosic biomass is composed by cellulose, hemicelluloses, and lignin. Cellulose is connected by hemicelluloses, and then covered by lignin. The complexity of chemical components and their interaction make the utilization and/or treatment of yard trimmings a big challenge. Therefore, pretreatment prior to further utilization is necessary to improve the amenities of yard trimmings to biodegradation.

Some pretreatment methods, such as alkali pretreatment (Cherosky, 2012) and wet oxidation pretreatment (Lissens et al., 2004), have been studied to reduce the recalcitrance of yard trimmings by increasing the accessibility of cellulose and
hemicellulose to enzymes and microbes, and to improve the methane production. However, NaOH pretreatment of yard waste showed no positive effect on methane yield, and wet oxidation pretreatment was not cost-effective because of the use of high temperature and oxygen. Biological pretreatment by using white rot fungal, has been proven effective in selectively degrading lignin and increasing enzymatic degradability of lignocellulosic biomass for ethanol production (Wan and Li 2010b). It is considered an environmentally friendly and low-cost pretreatment technique. Pretreatment of hardwood, corn stover, and rubberwood with a white rot fungus, Ceriporiopsis subvermispora was reported to significantly increase the glucose yield from enzymatic hydrolysis (Nazarpour et al., 2013; Wan and Li, 2010b). Amirta et al. (2006) found a 74-76% decrease in β-O-4 aryl ether linkages in the lignin after using C. subvermispora to pretreat Japanese cedar wood for 8 weeks with the presence of wheat bran. Since yard trimmings have similar compositions to other lignocellulosic biomass, white rot fungi pretreatment could be a promising strategy to reduce its recalcitrance and further improve its methane yield during the SS-AD process.

Therefore, the goal of this chapter is to test whether C. subvermispora could selectively degrade lignin and thus increase the methane yield from SS-AD of yard trimmings. Three objects were addressed, including 1) the effect of pretreatment on the degradation of cellulose and hemicellulose and lignin at different aeration modes and moisture content, 2) the effect of pretreatment on the methane yield of yard trimmings, 3) the effect of
pretreatment on the improvement of volatile solid (i.e., cellulose, hemicellulose) degradation during SS-AD.

3.2. Materials and Methods

3.2.1. Feedstock and fungus preparation

Yard trimmings were collected from the Ohio Agricultural Research and Development Center (OARDC) in Wooster, OH. After air-dried till moisture content less than 10%, yard trimmings were ground to pass through a screen sieve (0.5-inch open size) with a hammer mill (C.S. Bell Co., Tiffin, OH, USA). Samples were bagged and stored at ambient condition until used. The chemical composition characteristics of yard trimmings were shown in Table 3.1.

Table 3.1. Chemical composition of yard trimmings

<table>
<thead>
<tr>
<th>Chemical component</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td>30.77±0.91</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>15.87±0.48</td>
</tr>
<tr>
<td>Acid soluble lignin</td>
<td>0.57±0.06</td>
</tr>
<tr>
<td>Acid insoluble lignin</td>
<td>32.31±0.06</td>
</tr>
<tr>
<td>Extractives</td>
<td>9.56±0.28</td>
</tr>
<tr>
<td>Volatile solid</td>
<td>94.31±0.11</td>
</tr>
<tr>
<td>Total solid</td>
<td>98.94±0.15</td>
</tr>
</tbody>
</table>

The percentage of composites is based on dry weight, except for total solid.

The white rot fungus *Ceriporiopsis subvermispora* (ATCC 96608) was purchased from American Type Culture Collection (Manassas, VA, USA) and maintained on 2% (w/v) malt extract agar (MEA) plates at 4°C. The fungus was first cultured on 2% MEA at 28°C for 7 days and then 3 small pieces agar medium with fungus mycelium were
transferred into 20 mL of 2% malt extract liquid medium in 125-mLErlenmeyer flasks with cotton plugs (in the neck of flasks). After another-7 day cultivation at 28°C without agitation, the fungi were aseptically in a biosafety laminar hood (Labconco Corporation, Kansas City, MO, USA) harvested by dumping the spent medium, and resuspended in sterilized DI water. The fungus “solution” was then used as inoculum for pretreatment.

3.2.2. Fungal pretreatment

After 100g (dry basis) of yard trimmings were charged into 1-L glass bottles, deionized (DI) water was added to obtain desired moisture contents of 45%, 60% and 75%, respectively. Bottles with yard trimmings and DI water were autoclaved at 121°C for 30 min. After cooling, yard trimmings were inoculated with the prepared fungus “solution”. Each 1L bottle was inoculated with fungus “solution” harvested from the 125ml flask as described above with 20ml liquid medium with liquid part dumped. Pretreatment was carried out in a low-temperature incubator (Fisher Scientific, Hampton, NH, USA) at 28°C for 30 days without stirring. At each moisture content, three different aeration modes were applied, i.e., natural air (air allowed by cotton stoppers into reactors without mechanical aeration), 150±10ml/min forced air for 30min every 24h, and 150±10ml/min forced air for 15min every 12h. As shown in Table 3.2, two-factor full factor design (3 levels for each factor) was employed for pretreatment. Sterilized yard trimmings without inoculation were used as controls. After pretreatment, all samples were subjected to SS-AD to test the methane production. The dry matter loss and loss of cellulose,
hemicellulose and lignin due to pretreatment were also monitored (Equations 1 and 2).

All tests were performed in duplicate.

\[
\text{Dry matter loss} = \frac{\text{TS}^\text{Initial} - \text{TS}^\text{Final}}{\text{TS}^\text{Initial}} \times 100\% \quad (1)
\]

\[
\text{Chemical composition loss} = \frac{\text{Mass}^\text{Initial} - \text{Mass}^\text{Final}}{\text{Mass}^\text{Initial}} \times 100\% \quad (2)
\]

Table 3.2. Experimental design of fungal pretreatment

<table>
<thead>
<tr>
<th>Moisture content (%)</th>
<th>Aeration mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>Natural air</td>
</tr>
<tr>
<td>60</td>
<td>15min/12h</td>
</tr>
<tr>
<td>75</td>
<td>30min/24h</td>
</tr>
</tbody>
</table>

3.2.3. Solid-state anaerobic digestion

The inoculums for SS-AD were the effluent from a mesophilic liquid anaerobic digester (mainly fed with municipal wastewater) in Zanesville, OH, operated by the Quasar Energy Group. Effluent was kept in air-tight drums for storage at 4°C in a walk-in cooler. Before being used for inoculation, effluent was placed in 37°C chamber for 1 week to reactivate microbes. Due to its low TS, the effluent was dewatered by centrifugation to achieve a TS of approx. 11%, and VS of 5.2% (dry basis).
In SS-AD, the ratio of feedstock to effluent (F/E ratio) (VS basis) was 4 which was chosen based on the preliminary test and the TS requirement (over 20%) in SS-AD. Feedstock for each reactor was 50g (VS basis), which means the VS of effluent should be 12.5g. The initial TS of all SS-AD reactors were set to 20%. DI water was used to adjust the TS to 20% when necessary. After well mixed, the mixture was loaded in a 1-L glass reactor sealed with a robber stopper. All AD reactors were connected to 5-L gas bags (CEL scientic Tedlar gas bag, Santa Fe Springs, CA, USA) for biogas collection. Reactors were incubated in a walk-in incubation room at 37±1 °C for 45 days. Biogas compositions and volume were determined every day during the first 5 days, and day 7, 10, 15, 20, 30, and 40.

3.2.4. Analytical methods

TS and VS of feedstock and effluent were measured according to the Standard Methods for the Examination of Water and Wastewater (APHA, 2005). The structural carbohydrate (cellulose and hemicelluloses) and lignin of yard trimmings and digestate of SS-AD were measured by following the method developed by NREL (Sluiter et al., 2008).

300.0±10.0 mg (dry basis) yard trimmings were first hydrolyzed by 3.00±0.01ml 72% sulfuric acid for 60±5 min in pressure tubes placed in 30±3°C water bath. During this hydrolysis process, using a stir rod, the sample was stirred every 5 to 10 min. After the
60-min hydrolysis, the tubes were removed from water bath and the acid was diluted to 4% by adding $84.00 \pm 0.04$ml deionized water. The Teflon caps were screwed on securely and the sample was mixed completely. The tubes were placed in autoclave at 121°C for another one hour’s hydrolysis. Along with the samples, a set of sugar recovery standards (SRS) with 4% sulfuric acid were also placed into autoclave. The SRS were used to correct the losses due to destruction of sugars during this dilute acid hydrolysis.

After the dilute acid hydrolysis, around 20 mL samples were taken out for neutralization till pH reached 5-6 with calcium carbonate. The sample for HPLC analysis was prepared by passing the neutralized sample through a 0.2 µm filter into an autosampler vial. Cellulose and hemicelluloses in feedstocks were hydrolyzed into monomeric sugars through this two-step acid hydrolysis, which were measured by a high pressure liquid chromatography (HPLC). The content of cellulose was calculated from glucose while hemicellulose was calculated by the sum of xylose, arabinose, galactose, and mannose, using an hydro correction of 0.90 for C-6 sugars and 0.88 for C-5 sugars.

Lignin is the sum of acid-soluble lignin and acid-insoluble lignin. The acid-soluble lignin was measured by UV-Vis spectroscopy at wavelength of 240 nm. The sample for this test was aliquot after the diluted acid hydrolysis. 4% sulfuric acid was used as blank. The aliquot should be diluted if necessary to make the absorbance lies in the range of 0.7-1.0. The acid-insoluble lignin was measured by gravimetric analysis. Filtering crucibles were first autoclaved for at least 1 hour. After cooling, the autoclaved filtering crucibles were
weighed. The diluted acid hydrolysis solution was vacuum filtered through one the weighed filtering crucibles. The filtrate was captured in a filtering flask. All the remaining solids were quantitatively transferred by using deionized water out of the pressure tube into the filter crucible. The solids were rinsed with a minimum of 50 mL fresh deionized water. The crucible and acid insoluble residue were dried at 105 ± 3°C until a constant weight was achieved. The samples were removed from oven and cooled in a desiccator. Crucible and dry residue were weighed. Then the crucibles and residue were placed in the muffle furnace at 575 ± 25°C for 6 hours. The crucibles and ash were removed from the furnace into a desiccator and were weighed after cooling down.

The separation column used for HPLC (Shimadzu LC-20, Shimadzu, Columbia, MD, USA) used for sugar analysis was an Aminex HPX-87P (Bio-Rad Laboratories, Berkeley, CA, USA), HPLC grade water was used as the mobile phase at a flow-rate 0.3 mL/min. The sugar components were detected by a refractive index detector (RID). The temperatures of the column and RID were maintained at 65°C and 55°C, respectively.

The volume of biogas collected in gas bags was measured with a drum-type gas meter (Ritter, TG 5, Germany) by connecting the biogas collection bag with the drum and pumping the biogas out, the volume of which was reflected by the dropping of liquid level. The composition of biogas (CO₂, CH₄, N₂, and O₂) was analyzed by a gas chromatography (GC) (Agilent Technologies, HP 6890, DE, USA) equipped with a 10-ft stainless steel column 45/60 Molecular Sieve 13X and a thermal conductivity detector.
(TCD). Helium was used as a carried gas at flow rate of 5.2 mL/min. The temperature of the detector was set at 200°C. The temperature of the column oven was initially programmed to be 40°C for 4 min, then rise at 20°C/min to 60°C and remained at 60°C for 5 min.

3.2.5. Data analysis

Statistical significance was determined by analysis of variance (ANOVA, $\alpha=0.05$) using Minitab software (Version 16, Minitab, Inc., State College, PA, USA) with $p_{\text{critical}}=0.05$. Multiple comparisons were performed with Tukey test.

3.3. Results and Discussion

3.3.1. Degradation of yard trimmings by fungal pretreatment

The effect of moisture content and aeration mode varied in different responses (e.g., dry matter loss, cellulose, hemicellulose and lignin loss) (Figure 3.1). The treatment condition with natural aeration and moisture content of 60% resulted in the highest dry matter loss, and loss of cellulose, hemicellulose, and lignin.

The dry matter loss during fungal pretreatment process was as much as 12.45%, e.g., the yard trimmings pretreated at 60% moisture content with natural aeration (Figure 3.1a). While in the control group (autoclaved yard trimmings without fungal inoculation), the dry matter loss was as low as 1.35% (Data not shown). At moisture content of 45%, 15 min/12 aeration had the highest dry matter loss (9.5%), while natural aeration achieved
the highest dry matter loss at moisture content of 60% and 75%. Our results are similar to those from Wan and Li (2011) who also found *C. subvermispora* could effectively degrade hardwood chips with a dry matter loss around 10% in 18-day inoculation period. Amirta et al. (2006) obtained similar dry matter loss when applying the same fungal species but different strains for Japanese cedar wood pretreatment. Dry matter losses ranging from 13.2 to 15.9% were achieved after 8-week pretreatment with wheat bran as an additive.
Figure 3.1. Effect of moisture content and aeration mode on the degradation of yard trimmings during a 30-day fungal pretreatment: (a) dry mass loss; (b) cellulose loss; (c) hemicellulose loss; and (d) lignin loss.
Figure 3.1 Continued

(a) Hemicellulose loss (%) by aeration mode and duration.
(b) Lignin loss (%) by aeration mode and duration.
(c) Hemicellulose loss (%) for different aeration modes and durations.
(d) Lignin loss (%) for different aeration modes and durations.

Aeration mode

45%
60%
75%
The cellulose loss during fungal pretreatment under all the conditions is not significantly ($p>0.05$) different from cellulose loss (3.4%) of control (autoclave only, data not shown) (Figure 3.1b). Cellulose loss of yard trimmings was up to 7.4% (moisture content of 60% under natural aeration), which was much lower than hemicellulose (up to 27.6%) and lignin (up to 20.9%) loss (Figures 3.1c, d). Such results are likely due to the fact that *C. subvermispora* preferentially degrades hemicelluloses and lignin, as compared to cellulose. Previous studies showed that during the cultivation of *C. subvermispora*, there was no detectable cellulolytic activity in the medium as determined by using filter paper strips as substrate, while there was considerable amounts of xylanase, MnP, and laccase activity (Wan and Li, 2010a). Xylanase is responsible for the degradation of xylose, which is the main component of hemicellulose, and MnP and laccase are the two main lignin degradation enzymes. The presence of hemicellulose and lignin degradation enzymes but not cellulases could explain the selective degradation activity of *C. subvermispora*. Another study on *C. subvermispora* pretreatment of lignocellulosic biomass by Wan and Li (2011) confirmed that *C. subvermispora* preferentially degrades hemicellulose and lignin as compared to cellulose. In this study, cellulose loss was less than 5%, while the loss of hemicellulose and lignin reached over 15% in the fungal pretreatment of hardwood chips. In our test, the loss of the major substrate for methane production in SS-AD was not significant during fungal pretreatment.

For the loss of hemicellulose, pretreatment in natural aerated flask showed higher degradation as compared with other two aeration modes at all three moisture content
levels. When the aeration mode was changed from natural aeration to 30 min/24h, the hemicellulose loss decreased about 65% at moisture content of 60%. With the same aeration mode, moisture content of 60% had achieved the highest hemicellulose loss (Figure 3.1c). For example, under natural aeration, the hemicellulose loss at moisture content of 60% was 60% and 84% higher than that at moisture content of 75% and 45%, respectively. The impact of hemicellulose loss during fungal pretreatment on overall methane yield during AD was evaluated in the following section.

Compared to fungal pretreatment with natural aeration at moisture content of 60% and 75%, lower lignin loss was obtained at 15min/12h followed by 30min/24h when the moisture content was 60% or 75%. For example, aeration for 30 min/24h led to a significant decrease in lignin loss by 60% compared with aeration of natural air at moisture content 60%. (Figure 3.1d) When moisture content decreased to 45%, lignin loss with aeration of 15 min/12h was higher than that with other aeration modes. Under natural aeration and 30 min/24h, pretreatment of yard trimmings at moisture content of 60% and 75% had higher lignin loss than that at moisture content of 45%; while there was no significant difference between moisture content of 60% and 75%. Aerated under 15min/12h, the lignin loss decreased with the increase of moisture content from 45 to 75%. Pretreatment with moisture content of 45% and 60% had no significant difference in lignin loss between each other. Wan and Li (2010b) reported that the optimal moisture content for C. subvermispora in the pretreatment of corn stover was 75%. This is in agreement with other reports that moisture content ranging from 70% to 80% was the
optimal level for lignin loss. The different optimal moisture content for lignin loss found in this thesis could be attributed to the difference of feedstock and/or aeration mode. Compared with corn stover, yard trimmings have less ability to absorb water and are more resistant to biodegradation. In addition, aeration mode (e.g., aeration frequency and flow rate) and moisture content could significantly affect aeration efficiency, which could influence the activity of fungus and pretreatment effect. Statistical analysis by two-way ANOVA (Table 3.3) shows that moisture content and aeration mode are significant factors affecting lignin loss during pretreatment, with $p$ value of 0.003 and 0.000, respectively. The interaction between moisture content and aeration mode also significantly affects lignin loss with $p$ value of 0.003.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content</td>
<td>2</td>
<td>258.07</td>
<td>129.037</td>
<td>7.25</td>
<td>0.003</td>
</tr>
<tr>
<td>Aeration mode</td>
<td>2</td>
<td>695.80</td>
<td>347.898</td>
<td>19.54</td>
<td>0.000</td>
</tr>
<tr>
<td>Interaction</td>
<td>4</td>
<td>369.65</td>
<td>92.411</td>
<td>5.19</td>
<td>0.003</td>
</tr>
<tr>
<td>Error</td>
<td>27</td>
<td>480.79</td>
<td>17.807</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>1804.30</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.3.2. Biogas production in SS-AD of pretreated yard trimmings

Overall, pretreatment improved the methane yield of yard trimmings by 33 to 154%, and moisture content and aeration mode in pretreatment had substantial effects on the methane yield (Figure 3.2). For yard trimmings pretreated under moisture content of 60% and 75%, the methane yield decreased significantly when the aeration mode changed.
from natural air to 15 min/12 h and 30 min/24 h. However, the aeration of 15 min/12 h achieved the highest methane yield at moisture content of 45%. When comparing the effect of moisture content on the methane yield, we can find that moisture content of 60% had the highest methane yield followed by moisture content of 45% and then 75% under natural aeration. For the other two aeration modes, the methane yield decreased with the increase of moisture content from 45 to 75%, especially the moisture content had little effect on the methane yield with the aeration mode of 30 min/24 h. Preliminary data showed that continuous aeration could cause the drop of moisture content. So, aeration of 15min/12h and 30min/24h were investigated to avoid the drop of moisture content. During the aeration process, rubber stopper with one inlet and one outlet tubes were used. The lower methane yield of yard trimmings pretreated under these two aeration modes might be caused by the insufficient oxygen, allowed by the tubes in rubber stoppers. Further research about aeration during fungal pretreatment process is needed. Among all treatments, yard trimmings fungally pretreated at moisture content of 60% with natural aeration had the highest cumulative methane yield of 44.6L/kg VS (original yard trimmings) (in this thesis, the methane yield is all based on the volatile solid of original yard trimmings) after 40-day SS-AD, which is over 2 times higher than the methane yield (21.2 L/kg VS) of the autoclaved yard trimmings without fungal pretreatment and the methane yield (17.6 L/kg VS) of the raw yard trimmings without fungal pretreatment (Figure 3.2). The highest methane yield from fungal pretreated yard trimmings is about 18.3% of the theory methane potential of original yard trimmings, while the methane
yield from yard trimmings without pretreatment is about 8.3% of the theory methane potential of original yard trimmings.

![Cumulative methane yield from 40-day SS-AD of yard trimmings pretreated under different moisture contents and aeration modes (Data for the autoclaved and raw materials are not shown)](chart)

**Figure 3.2. Cumulative methane yield from 40-day SS-AD of yard trimmings pretreated under different moisture contents and aeration modes (Data for the autoclaved and raw materials are not shown)**

Sterilization (e.g., autoclave) is usually required in fungal pretreatment of lignocellulosic biomass since fungus is sensitive to contamination (our preliminary tests). In our studies, autoclaving was also tested as a pretreatment method; however, autoclaving without inoculation of *C. subvermispora* did not significantly improve the methane yield (Table 3.4). Based on multiple comparisons among different treatments, it can be concluded that natural aeration generally had better effects than the other two aeration modes on methane yield; and 45% moisture content achieved higher methane yield than moisture content of 60% and 75% (Table 3.4). Pretreatment at 60% moisture content with nature aeration led to the highest methane yield, however there was no significant difference
between 15min/12h or natural aeration at 45% moisture content. Therefore, the pretreatment with 45% moisture content and natural aeration was the best choice in lab scale test.

Table 3.4. Tukey multiple comparisons of cumulative methane yield from yard trimmings pretreated under different conditions

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>N</th>
<th>Mean</th>
<th>Grouping</th>
</tr>
</thead>
<tbody>
<tr>
<td>na-60%</td>
<td>2</td>
<td>44.62</td>
<td>A</td>
</tr>
<tr>
<td>12h-45%</td>
<td>2</td>
<td>41.10</td>
<td>A B</td>
</tr>
<tr>
<td>na-45%</td>
<td>2</td>
<td>40.30</td>
<td>A B C</td>
</tr>
<tr>
<td>12h-60%</td>
<td>2</td>
<td>34.98</td>
<td>B C</td>
</tr>
<tr>
<td>na-75%</td>
<td>2</td>
<td>32.58</td>
<td>CD</td>
</tr>
<tr>
<td>24h-45%</td>
<td>2</td>
<td>26.21</td>
<td>D E</td>
</tr>
<tr>
<td>12h-75%</td>
<td>2</td>
<td>25.26</td>
<td>D E F</td>
</tr>
<tr>
<td>24h-60%</td>
<td>2</td>
<td>24.61</td>
<td>D E F</td>
</tr>
<tr>
<td>24h-75%</td>
<td>2</td>
<td>23.48</td>
<td>E F</td>
</tr>
<tr>
<td>auto</td>
<td>2</td>
<td>21.17</td>
<td>E F</td>
</tr>
<tr>
<td>org</td>
<td>2</td>
<td>17.59</td>
<td>F</td>
</tr>
</tbody>
</table>

Groups that do not share a letter are significantly different (Tukey 95% Simultaneous Confidence Intervals) (“na” means “natural aeration”; “12h” means “15min/12h”; “24h” means “30min/24h”; “auto” means “autoclaved yard trimmings”; “org” means “original yard trimmings”; and 45%, 60%, and 75% mean the moisture contents).

In fungal pretreatment, aeration mode and moisture content are two significant factors affecting methane yield, as well as the interaction between both factors. (Table 3.5)

Table 3.5. Two-way ANOVA on the effect of moisture content and aeration mode on methane yield

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content</td>
<td>2</td>
<td>272.42</td>
<td>126.21</td>
<td>27.44</td>
<td>0.000</td>
</tr>
<tr>
<td>Aeration mode</td>
<td>2</td>
<td>635.23</td>
<td>317.616</td>
<td>63.97</td>
<td>0.000</td>
</tr>
<tr>
<td>Interaction</td>
<td>4</td>
<td>138.86</td>
<td>34.72</td>
<td>6.99</td>
<td>0.008</td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td>44.68</td>
<td>4.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>1091.20</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

50
According to the correlation between lignin degradation and methane yield, it was found that methane yield linearly increased with the lignin degradation by fungal pretreatment (Figure 3.3). With the increase of lignin degradation from 0 (raw material) to 21%, the methane yield was enhanced by 154% (from 17.6-44.6 L/kg VS). The results in our studies are consistent with the previous reports that indicated that the lower the lignin content (or the higher lignin loss), the higher yield of enzymatic hydrolysis, higher bioethanol yield, or biogas production was achieved (Liew et al., 2011; Wan and Li, 2010b). This is because the barrier of lignin is reduced and thus the availability of feedstock for microbes or enzymes increased. Take et al. (2006) found a negative correlation coefficient of -0.98 between methane volume and Klason lignin content, which is the high-molecular weight lignin inhibiting the hydrolysis process. Nazarpour et al. (2013) reported linear correlation between lignin degradation (by fungus of \textit{C. subvermispora}, \textit{T. versicolor} and the mixture of \textit{C. subvermispora} and \textit{T. versicolor}) and reducing sugar yield from enzymatic hydrolysis.
As shown in Figure 3.4 a, b, and c, the methane content of almost all the SS-AD reactors reached to stable levels after 21 days. There is no significant difference on the methane content among all conditions including fungal and autoclave pretreatment and raw material. The final methane content was approx. 60%, which indicates that all the reactors were “healthy”, and the lower methane yield was not caused by the inhibition of the AD process. In another word, the digestibility of feedstock is the main factor affecting the methane yield.
Figure 3.4. Effect of moisture content and aeration mode in fungal pretreatment on methane content during 40-day SS-AD. a) Natural aeration; b) 15 min/12 h; c) 30 min/24 h; d) comparison between fungal pretreatment, autoclave treatment and raw material on methane content.
Figure 3.4 Continued

**c**

Methane Content (%)

- 45%
- 60%
- 75%

Time (Day) 0 5 10 15 20 25 30 35 40

**d**

Methane Content (%)

- Natural aeration_60%
- Autoclaved
- ORG

Time (Day) 0 5 10 15 20 25 30 35 40
3.3.3. Loss of holocellulose during SS-AD

Loss of cellulose and hemicellulose during the 40-day SS-AD is shown in Figure 3.5. There was almost no loss of cellulose observed in both autoclaved yard trimmings and original yard trimmings (data not shown). This is consistent with results from Brown (2012) and Liew et al. (2011) who achieved cellulose loss of 0% and 6%, respectively, during 30-day SS-AD of original yard waste. Cellulose loss of fungal pretreated yard trimmings (60% moisture content under natural aeration) reached 15.04%, which could be ascribed to the lignin loss by fungal pretreatment, leading to removal of the structural obstacles and making cellulose more accessible to enzymes and microbes.

There was little loss of hemicellulose in SS-AD of original yard trimmings. A 5.7% and 16.7% loss of hemicelluloses was observed in SS-AD of fungal pretreated (60% moisture content under natural aeration) and autoclaved yard trimmings. Brown (2012) reported no loss of hemicelluloses during a 30-day SS-AD of original yard wastes and Liew (2011) found a 7% loss of hemicelluloses in original yard wastes during 30-day SS-AD with F/E ratio of 2. A higher loss of hemicellulose was observed in autoclaved yard trimmings. This might be attributed to high temperature and the catalysis of water during autoclave. During autoclaving process, the high temperature might solubilize hemicellulose (Takara and Khanal, 2008). The solubilized hemicellulose was easy to be utilized by microbes in AD reactors. For the fungal pretreated yard trimmings, which also go through the autoclave process, the solubilized hemicellulose is utilized by \textit{C. subvermispora} during the fungal pretreatment process. After fungal pretreatment, no easily degradable
hemicellulose may be available for the SS-AD process, so there is a lower loss of hemicellulose in SS-AD of fungal pretreated yard trimmings than that in SS-AD of autoclaved yard trimmings. Even though there was little loss of cellulose and hemicelluloses observed, the original yard trimmings still had cumulative methane yield of 17 L/kg VS. This is probably due to the utilization extractives (e.g., soluble carbohydrates, wax, and other small molecules) by SS-AD to produce biogas. A previous study conducted by Liew (2011) indicated that loss of cellulose and hemicellulose was quite low, while the degradation of extractives could reach over 50% during 30-day SS-AD of yard waste.
3.4. Conclusions

Fungal pretreatment by *C. subvermispora* preferentially degraded lignin. The greatest lignin loss observed was 20.9%. Pretreatment of yard trimmings by *C. subvermispora* increased the methane yield from SS-AD as much as 154%. The greatest methane yield from 40-day SS-AD of fungal pretreated yard trimmings was 44.6 L/kg VS (original yard trimmings) and corresponded to fungal pretreated yard trimmings with a moisture content...
of 60% and natural aeration. The methane enhancement was linearly correlated with the extent of lignin loss during fungal pretreatment.

For the lab scale test, natural aeration provided enough oxygen for the growth of *C. subvermispora* during pretreatment. However, mechanical ventilation might be necessary for a scaled up fungal pretreatment system.
Chapter 4. Enhancement of Methane Yield from Solid-state Anaerobic Digestion of Yard Trimmings by Composting

Composting could be used as a biological pretreatment to improve methane yield from anaerobic digestion of high-lignin content lignocellulosic biomass such as yard trimmings, considering the fact that lignin mineralization happened during composting process. A simulated composting was performed on the mixture of yard trimmings and effluent from liquid anaerobic digester (AD) with feedstock to effluent (F/E) ratios of 19 and 23. After 30 days of composting, the yard trimmings were digested for 30 days under mesopilic solid-state AD conditions at a F/E ratio of 3. There was no lignin loss observed during composting under either F/E ratio. While the loss of both cellulose and hemicellulose was around 20%. The cumulative methane yield of composted yard trimmings (23.3 L/kg VS for F/E ratio of 19, and 21.7 L/kg VS for F/E ratio of 23) was significantly lower than that of the uncomposted yard trimmings (36.1 L/kg VS). No cellulose or hemicellulose loss was observed in composted yard trimmings during 30-day SS-AD, while a loss of 7.7% and 11.5% was observed for cellulose and hemicellulose in uncomposted yard trimmings, respectively. It was shown in this study that composting cannot enhance methane production from SS-AD of yard trimmings.
4.1 Introduction

The disposal of yard trimmings, the main component of municipal solid waste (MSW), is an environmental issue. Considering the high percentage (50%) of holocellulose, yard trimmings can be used for biogas production through solid-state anaerobic digestion (SS-AD). By utilization of AD in disposal of yard trimmings, both pollution control and energy production could be achieved. Like other types of lignocellulosic biomass, yard trimmings have a high lignin content. When combined with lignin, holocellulose becomes less degradable or even completely refractory (Tong et al., 1990). The complex structure formed by lignin and holocellulose makes yard trimmings not easily available for microbes and enzymes, leading to a low methane yield. Therefore, a pretreatment step of yard trimmings prior to SS-AD is necessary to improve methane production.

Previous studies have shown that yard trimmings could be a suitable feedstock for biogas production via AD process (Brown, 2012). However, the premise is yard trimmings need to be pretreated to improve its biodegradability in AD (Liew, 2011). Yard trimmings used in this study were composed of 31% cellulose, 16% hemicellulose and 33% lignin. The pretreatment focus is delignification, which could effectively improve biogas yield of yard trimmings. For example, fungal pretreatment can effectively enhance biogas production in SS-AD by removing lignin content to certain extent (Chapter 3). However, fungal pretreatment requires moisture adjustment and autoclaving, which might make fungal pretreatment costly.
Therefore, alternative biological pretreatments that do not require extra water addition or autoclaving may be more economical for SS-AD. Based on these considerations, composting could be an alternative biological pretreatment technique to fungal pretreatment because composting can degrade lignin without the need of autoclave. The effluent from liquid-AD (L-AD) could be an alternative water resource to meet the moisture requirement for yard trimmings composting. Composting as such could help address the issue of L-AD effluent disposal.

Based on the report from the International Energy Agency (IEA, 2008), there are 237 commercial digesters worldwide, with the capacity of 3000 to 497,000 tons of waste annually for each plant. Most digesters are L-AD systems. The effluent from such L-AD digesters is an issue for many commercial plants. Since L-AD effluent has a high moisture content and a large amount of ammonium and phosphate. If the effluent is not from a thermophilic digester, pathogens in the effluent are also a concern (Lei et al., 2007; Mata-Alvarez et al., 2000). Therefore, using L-AD effluent as a composting amendent with yard trimming could not only improve the composting treatment, but also address the disposal problem of L-AD effluent.

During composting, ligninolytic microbes such as fungi and ascomycotina can degrade lignin (Tuomela et al., 2000). Lignin is the most important factor impeding the biodegradation of lignocellulosic biomass (Tong et al., 1990). Therefore, the removal of lignin during the composting process might facilitate methane production in the
following SS-AD of composted yard trimmings. The high nitrogen content in effluent can be used to adjust the C/N ratio for composting as yard trimmings alone have a high C/N ratio (68.7).

Among the four phases of composting (mesophilic phase, thermophilic phase, cooling phase, and maturation phase), thermophilic phase is believed to play an important role in lignin degradation. Tomati et al. (1995) and Galli et al. (1997) found that a 70% degradation of lignin during the 30-day thermophilic phase, when the mixture of olive-mill wastewater and wheat straw with an initial C/N ratio of 35 was composted. During the cooling and maturation phases, humification happened to the soluble lignin with low molecular weight, but no lignin degradation was detected. In the research carried out by Horwath and Elliott (1996), component decomposition of ryegrass straw during mesophilic incubation and a staggered thermophilic incubation was investigated. The results showed that thermophilic condition achieved higher lignin degradation compared with mesophilic condition; but the degradation of lignin under both thermophilic and mesophilic conditions was lower than cellulose degradation. Composting prior to AD was conducted to improve biogas yield of OFMSW (Brummeler and Koster, 1990; Charles et al., 2009). It was found that composting removed easily degradable compounds of OFMSW so as to reduce the risk of acidification during the following AD and improve biogas yield. However, there are no reports on composting of lignocellulosic biomass as a lignin degradation method to improve methane yield from AD.
Therefore, the objective of this chapter is to test whether the thermophilic phase of composting with the addition of L-AD effluent could increase the digestibility of yard trimmings and thus improve the methane yield of composted yard trimmings in SS-AD process.

4.2 Materials and Methods

4.2.1 Materials

Yard trimmings were collected from the Ohio Agricultural Research and Development Center (OARDC) campus in Wooster, OH. After air-dried till moisture content less than 10%, yard trimmings were ground to pass through a 0.5-inch screen with a hammer mill (C.S. Bell Co., Tiffin, OH, USA). The compositions of yard trimmings included 30.8% cellulose, 15.9% hemicelluloses and 32.9% lignin. Herein, lignin consists of 0.6% acid-soluble lignin and 32.3% acid-insoluble lignin. The chemical compositions were all total solid (TS) based unless specified, otherwise. The TS of yard trimmings was 94.3%, and the volatile solid (VS) was 98.9% based on TS. The carbon/nitrogen (C/N) ratio of yard trimmings was 68.7 (based on total weight). Yard trimming particles were bagged and stored in the composting building at OARDC under ambient conditions until used.

The effluent used to mix together with yard trimmings for composting was obtained from a mesophilic liquid anaerobic digester mainly fed with food waste in Zanesville, OH, operated by the Quasar Energy Group. Effluent was kept in air-tight drums at 4°C in a walk-in cooler. The TS and VS of the effluent were 7.3% (based on wet weight) and 52.2% (based on TS), respectively. The C/N ratio of the effluent was 2.9 (based on total weight).
4.2.2 Composting of the mixture of yard trimmings and liquid AD effluent

Yard trimmings and effluent were mixed at two different feedstock/effluent (F/E) (VS basis) ratios of 19 and 23 corresponding to the moisture contents of 50%-60%. Yard trimmings in each 1-L glass reactor were 150g (total weight) and certain amount of effluent was added to reactors according to the designated F/E ratios. DI water was used adjust the moisture contents of all reactors to the same levels. Reactors were sealed with cotton balls and placed in an incubator (Fisher Scientific, Hampton, NH, USA) at 45°C for 30 days. During the composting process, there was a pan of water in the incubator to keep moisture content constant in the reactors. All tests were performed in duplicate.

4.2.3 Solid-state anaerobic digestion of composted yard trimmings

The effluent used as inocula for SS-AD was from a mesophilic anaerobic digester mainly fed with municipal wastewater in Akron, OH, operated by the KB Compost Services Inc. Effluent was kept in air-tight drums at 4°C in a walk-in cooler. Before the inoculation, effluent was placed in a chamber at 37°C for 1 week to reanimate microbes. The TS and VS of the effluent were 10% (wet basis) and 59.3% (dry basis), respectively.

For SS-AD reactors, feedstock/effluent (F/E) (VS basis) ratio of 3 was chosen to make the TS in reactors over 20% to reach solid state AD. Feedstock for each reactor was 50g (VS basis), which means the VS of effluent should be 16.7g. The initial TS of all SS-AD reactors were set to 21.3%. DI water was used to adjust the TS to 21.3% as necessary.
After mixing, the mixture was transferred into 1-L glass AD reactors plugged with a robber stopper and connected to 5-L gas bags (CEL scientic Tedlar gas bag, Santa Fe Springs, CA, USA) for biogas collection. Reactors were incubated in a walk-in incubator at 37±1 °C for 30 days. Biogas compositions and volume were measured every day during the first 5 days, and day 7, 10, 15, 20, and 30. All tests were performed in duplicate.

![Diagram of composting and SS-AD process]

Figure 4.1. Process for composting and SS-AD

4.2.4 Analytical methods

TS and VS of feedstock were measured before and after composting according to the Standard Methods for the Examination of Water and Wastewater (APHA, 2005), as well as the TS and VS of effluent before AD, and digestate after AD.
The structural carbohydrates (cellulose and hemicelluloses) and lignin of yard trimmings (before and after composting) and digestate of SS-AD were measured following a NREL method (Sluiter et al., 2008) with a few modifications. The detail of the analytical methods is stated in section 3.2.4.

4.2.5 Statistical analysis

Statistical significance was determined by analysis of variance (ANOVA, $\alpha=0.05$) using Minitab software (Version 16, Minitab, Inc., State College, PA, USA) with $p_{\text{critical}}=0.05$. Multiple comparisons were performed with Tukey test.

4.3 Results and Discussion

4.3.1 Degradation of yard trimmings during composting

The loss of different components and dry matter loss during a 30-day composting is showed in Figure 4.2. Composting under F/E=23 had higher loss of all the three components (cellulose, hemicelluloses and lignin) and dry matter loss than that under F/E=19. However, there was no statistically significant difference in the loss of these components with an exception of hemicelluloses and lignin.

During the composting process, the dry matter loss increased by 31% with the increase of F/E ratio from 19 to 23. Loss of cellulose reached to 19.5% (F/E=19) and 21.7% (F/E=23), both of which were higher than that of other components (hemicellulose and lignin). Horwath and Elliott (1996) found the similar result that cellulose had the largest
loss among all the components in ryegrass straw during a staggered thermophilic composting (a simulation of field composting). Following the degradation of cellulose, hemicellulose had the second highest loss among the main components, reaching to 14.1% and 19.4% under F/E=19 and 23, respectively.

![Bar chart showing loss of different components in composting]

**Figure 4.2. Degradation of yard trimmings during composting**

No remarkable lignin loss was observed under either F/E ratio. Horwath and Elliott (1996) reported that the loss of lignin was the slowest of all three components. But, a 29% decrease of lignin was obtained in a 45-day staggered high-temperature treatment, with 50°C between days 6-25 and 31-40. Tomati et al. (1995) also found a 70% reduction of lignin during the composting of olive-mill wastewaters and wheat straw, which mainly happened in the thermophilic phase lasting for 30 days. The possible reason for little loss
of lignin in this study could be insufficient aeration. During the composting process, no extra air was blown into reactors except for naturally air exchanging through the cotton stopper in the neck of reactors. Tomati et al. (1995) found a dramatic increase of oxygen consumption along with the increase of temperature. Oxygen consumption reached the maximum of 110 mmol h\(^{-1}\) kg\(^{-1}\) (dry weight) around day 15, which was part of the thermophilic phase. It was in this phase that the major degradation of lignin was detected. Therefore, sufficient air might be necessary for the loss of lignin. Because lignin degradation by ligninolytic enzymes is an oxidation process, oxygen plays an important role during this process. For example, during the single-oxidations of phenolic compounds in lignin catalyzed by laccases, O\(_2\) is reduced to H\(_2\)O (Chen et al., 2010). As a result, degradation of lignin might be more sensitive to the lack of oxygen than that of cellulose and hemicelluloses. According to Horwath and Elliott (1996), the degradation of cellulose could reach over 60% in the simulated composting process, while only around 20% degradation of cellulose observed in this study. It seems that the microbial cellulolytic activity in composting was impaired by insufficient aeration.

4.3.2 Biogas production in SS-AD of composted yard trimmings

Among the three groups, the control group had the highest cumulative methane yield of 36.1 L/kg VS, compared with groups of composted yard trimmings under F/E=19 (23.3 L/kg VS) and F/E=23 (21.7 L/kg VS) (Figure 4.3). The methane yield from the two composted groups are about 9.5% and 8.6% of the theory methane potential of yard trimmings, respectively, while the control group reached 17.0%. Liew (2011) reported
similar result of around 40 L/kg VS methane yield from 30-day SS-AD conducted at F/E ratio of 3. As discussed in Chapter 3, the methane production in the control group mainly derived from extractives and partly from the degradation of cellulose and hemicelluloses (Liew, 2011). During the composting process, however, the easily degradable extractives and amorphous cellulose (low crystallinity) were metabolized for cell growth and maintenance of cell activity, leaving the rigid high crystallinity region of cellulose (Zheng et al., submitted). The methane produced in two composted groups might be attributed to the large amount of microbial cells generated during composting process (Ryckeboer et al., 2003). Most of these microbes during composting are aerobic, and might not live in the SS-AD process. (Tuomela et al., 2000) The dissolving of these cells might release easily degradable substrates for the production of methane.
As shown in Figure 4.4, daily methane yield of both composted and control groups had the similar trend. The daily methane yield firstly peaked at day 2 and 3 for composted and original yard trimmings, respectively. Therefore, the composting pretreatment process did not change the trend of daily methane yield, even though it reduced the daily methane yield significantly compared with the control group.
Figure 4.4. Methane yield at every determination

The methane content of three different groups had the same trend (Figure 4.5). Methane content increased rapidly to approx. 50% in the first 5 days and stabilized at around 55% after about day 10, indicating the low methane yield was not caused by the failure of AD reactors. No significant difference was observed on methane content during stabilized stage between composted and control groups. Therefore, composting had little effect on methane content.
4.3.3 Loss of holocellulose during SS-AD of composted yard trimmings

Little loss of cellulose and hemicellulose was observed in the SS-AD of both composted yard trimmings (F/E=19 and 23) (Figure 4.6a, b). As mentioned in Section 4.3.2, it could be because the easily degradable amorphous cellulose and extractives were consumed during composting and composted residual became recalcitrant to biodegradation such as SS-AD. In the control group, 7.8% and 11.5% loss of cellulose and hemicelluloses were observed, respectively. This is consistent with the results reported by Liew (2011) who found the loss of cellulose and hemicellulose was 6% and 7%, respectively, during 30-day SS-AD. Liew (2011) also indicated that over 50% extractive was degraded during 30-day SS-AD. Hence, the methane produced from raw yard trimmings might mainly
derive from extractives, although loss of cellulose and hemicellulose also occurred. The methane produced in two composted groups might be ascribed to the large amount of debris of microbial cells generated during composting process (Ryckeboer et al., 2003). Most of these microbes during composting are aerobic, and might not be alive in the SS-AD process (Tuomela et al., 2000), thus served as substrate for the production of methane.

**Table 4.1. Degradation of cellulose and hemicellulose during SS-AD**

<table>
<thead>
<tr>
<th>Cellulose</th>
<th>Hemicellulose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mass</strong></td>
<td><strong>Mass</strong></td>
</tr>
<tr>
<td>Initial (g)</td>
<td>Final (g)</td>
</tr>
<tr>
<td>F/E=19</td>
<td>13.99</td>
</tr>
<tr>
<td>F/E=23</td>
<td>13.46</td>
</tr>
<tr>
<td>Control</td>
<td>16.24</td>
</tr>
</tbody>
</table>

**4.4 Conclusions**

Composting was conducted as a biological pretreatment technique in an effort to improve the methane yield of yard trimmings. The cumulative methane yield of composted yard trimmings was significantly lower than that of the original yard trimmings. Composting of the mixture of yard trimmings and AD effluent (at 45 °C under both F/E ratios of 19 and 23 for 30 days) did not result in significant degradation of lignin. Both the degradation of cellulose and hemicellulose, however, were around 20%. No cellulose and hemicelluloses loss is observed in composted yard trimmings during a 30-day SS-AD, while a loss of 7.8% and 11.5% was observed for cellulose and hemicelluloses, respectively, in original yard trimmings.
Chapter 5. Conclusions and Suggestions for Future Research

Fungal pretreatment by Ceriporiopsis subvermispora was shown to be effective in selectively delignifying and enhancing methane yield from SS-AD of yard trimmings. It was found that inoculation with C. subvermispora led to the selective loss of lignin of up to 20.9%, with only a limited amount of cellulose loss (around 7%). The greatest methane yield was over 2 times higher than the control groups. Both aeration mode and moisture content had significant effects on fungal pretreatment and methane yield during SS-AD. Both the highest methane yield and lignin degradation were obtained at moisture content of 60% with natural aeration. There existed a linear correlation between lignin degradation and methane yield. In lab scale, natural aeration is enough to provide sufficient oxygen for the fungal pretreatment. However, mechanical ventilation might be necessary for larger scale fungal pretreatment system. Even though fungal pretreatment by C. subvermispora can improve methane yield of yard trimmings, autoclaving of yard trimmings is essential before the inoculation of this fungus. The autoclave step makes this pretreatment less economic for large scale systems. An alternative way to avoid autoclave needs further investigation.

A simulated thermophilic phase of composting was used for the pretreatment of yard trimmings, mixed with AD effluent. During the composting process, no remarkable lignin
degradation happened. The composting process had no positive effect on the methane yield from SS-AD in this study and therefore is not a promising area for further study.
Reference


