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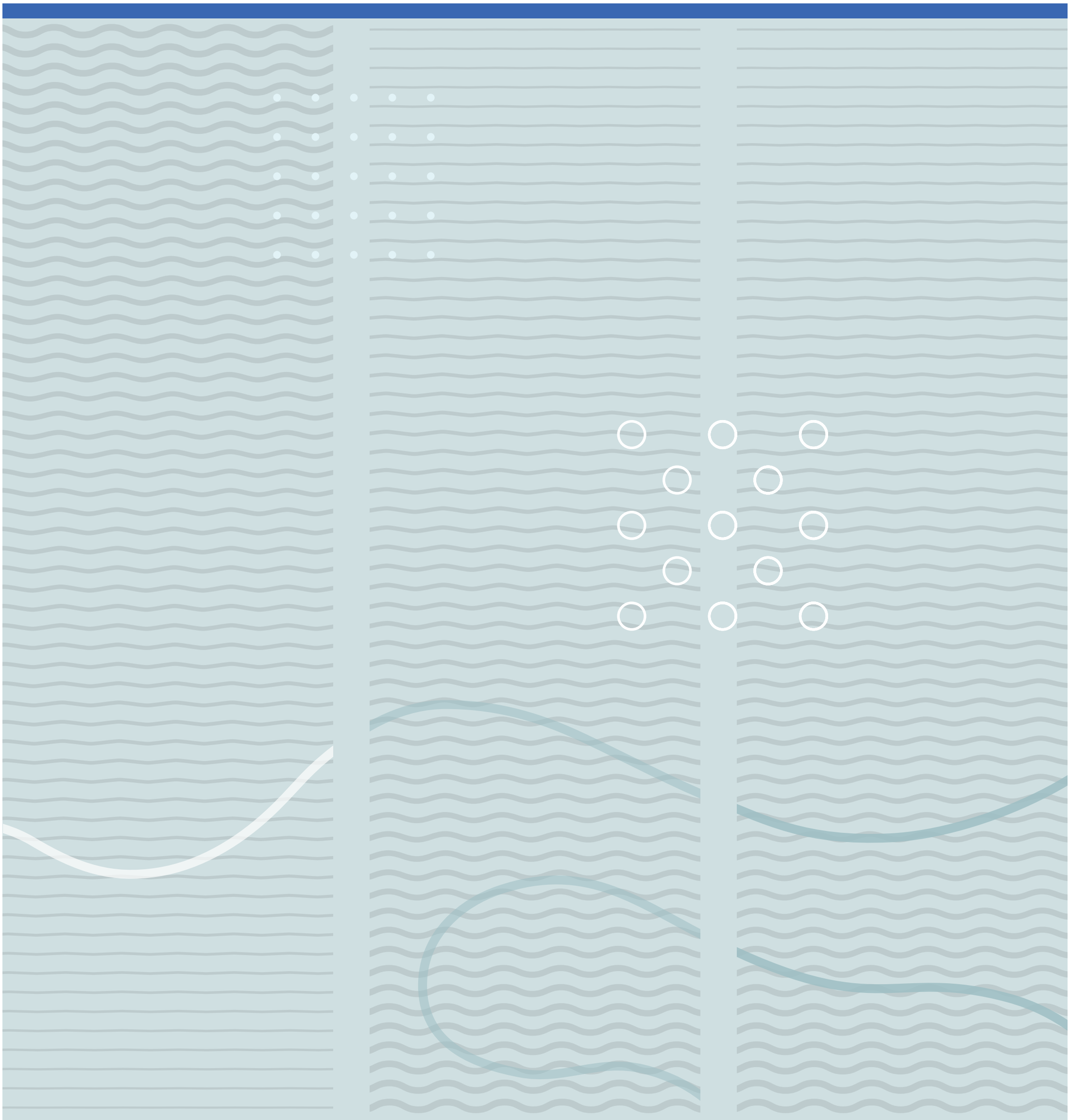
Nirmal Ghimire

Methane production from lignocellulosic residues



Nirmal Ghimire

Methane production from lignocellulosic residues

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Preface

This dissertation is submitted to the University of South-Eastern Norway (USN) in partial fulfilment of the requirements for the degree of Philosophiae Doctor (Ph.D). This work has been carried out under the supervision of Associate Professor Wenche Hennie Bergland and Professor Rune Bakke.

This dissertation consists of two parts. First part contains overview of the research project including literature review, method and materials, results and discussion and conclusion. Scientific articles, which are core part of the thesis, are included in the part II.

This research was accomplished in collaboration with RISE PFI AS. The main task of the PhD work carried out at USN was anaerobic digestion (AD) of waste streams produced during biochar production at RISE PFI AS, which provided AD feeds for the experiments.

Other external partners on this project were Norske Skog Saugbrugs, Ferroglobe, Cambi, Eramet Norway and Norwegian University of Science and Technology (NTNU). The work was done as a part of Norske Skog Saugbrugs innovation project Pyrogas co-funded by The Norwegian Research Council (EnergyX Programme).

I participated in two international conferences during the study period. Planned third international conference “8th International Conference on Sustainable Solid Waste Management”, Greece, 17-20 June 2020, was moved to 2021 due to COVID-19. I was also involved in other projects taking place at USN and elsewhere.

The review article (Paper 5) sent to Bioresource Technology has now been accepted and published.

Courses (**30 ECTS**) I attended during the PhD periods are as following:

- **Theory of Science and Ethics (D0611)**, USN (Bø) (**5 ECTS**)
- **Process of Analytical Technology (D0110)**, USN (Porsgrunn) (**5 ECTS**)

- **Microbial Ecology (BT8101)**, NTNU (Trondheim) (**9 ECTS**)
- **Sustainable Biomass Resources and Technology Pathways of Biogas and Biorefineries**, Aalborg University (Esbjerg) (**5 ECTS**)
- **Advanced Environmental Biotechnology**, Delft University of Technology (Delft) (**3 ECTS**)
- **Sustainability and Environmental Aspects in Biomass Production Systems**, Swedish University of Agricultural Sciences (Uppsala) (**3 ECTS**)

Nirmal Ghimire

Porsgrunn, January 2021

In the memory of ***Prof. Rune Bakke***

"When great trees fall,
rocks on distant hills shudder,
lions hunker down in tall grasses,
and even elephants
lumber after safety.

When great trees fall
in forests,
small things recoil into silence,
their senses
eroded beyond fear.

When great souls die,
the air around us becomes
light, rare, sterile.
We breathe, briefly.
Our eyes, briefly,
see with
a hurtful clarity.
Our memory, suddenly sharpened,
examines,
gnaws on kind words
unsaid,
promised walks
never taken.
Great souls die and
our reality, bound to
them, takes leave of us.
Our souls,
dependent upon their
nurture,
now shrink, wizened.
Our minds, formed
and informed by their
radiance,
fall away.
We are not so much maddened
as reduced to the unutterable ignorance
of dark, cold
caves.

And when great souls die,
after a period peace blooms,
slowly and always irregularly. Spaces fill
with a kind of
soothing electric vibration.
Our senses, restored, never
to be the same, whisper to us.
They existed. They existed.
We can be. Be and be
better. For they existed."

- Maya Angelou (1928-2014)

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I am grateful to my main supervisor Associate Professor Wenche Hennie Bergland for her immense support, guidance and motivation throughout the journey. I must add that she has supported me more than I ever could have hoped for. I am deeply indebted to her personal kindness. I am equally grateful to my co-supervisor Professor Rune Bakke for his continuous support and guidance since my master's degree. I will always be indebted to his trust on me which pushed me to do better. He had been my guardian on all fronts. I will always miss him.

I also want to thank Associate Professor Carlos Dinamarca for his invaluable suggestions during difficult times. I am thankful to Eshetu Janka for his help in running experiments smoothly and update on African politics. I am also thankful to Hildegunn Hegna Haugen and Frank Aarvak for keeping labs well-functioning throughout the project period. I would also like to thank Cornelis van der Wijst, Øyvind Eriksen and Kai Toven from RISE-PFI for their valuable co-operations. I am also thankful to master thesis students Jitendra Sah, Vibeke Bredvold and Zahra Nikbakht Kenarsari for their collaborations.

Finally, I am grateful to my parents who believed in me and motivated me to take this immense task.

Abstract

Aims

Biochar production by intermediate pyrolysis of renewable lignocellulosic biomass to replace traditional carbon material as a reducing agent and energy source in the metallurgical industries produces carbon rich waste streams viz., hemicellulose hydrolysate from hot water extraction (HWE) and aqueous pyrolysis liquid (APL) from pyrolysis requiring efficient treatment before discarding to enhance energy recovery and avoid environmental problems. Anaerobic digestion (AD), a robust biological process, was considered to treat these challenging organic waste streams individually or as co-digestion for enhanced energy recovery in the form of methane. AD of hydrolysate and APL, both individually and as co-digestion, was performed to study the effect of HWE and pyrolysis temperatures and biomass types on the methane yield. Effect of AD temperature and organic load (OL) on methane yield from Norway spruce hydrolysate was also studied.

Materials and methods

Air-dried wood chips of Norway spruce and birch were hot water extracted in two different conditions of 140 °C for 300 min and 170 °C for 90 min to produce hemicellulose rich hydrolysate to use as AD substrate. The wood chips (with or without HWE) were pyrolyzed at 550 °C or 400 °C to produce APL which was used as AD substrate. Both hydrolysate and APL were prepared and supplied by RISE-PFI, Trondheim, Norway.

The hydrolysates from HWE and the APL from pyrolysis were tested for bio-methane potential (BMP) during batch AD in an Automatic Methane Potential Test System II (AMPTS II, Bioprocess Control® Sweden AB). Syringe batch reactors were used to study the effect of OL on methane yield.

Simplified lab scale up flow anaerobic sludge bed (UASB) reactors of 345 mL working volume were used for mesophilic continuous AD of Norway spruce hydrolysates.

Results and discussions

Hydrolysate of Norway spruce and birch showed good biodegradability (ranging from 69 to 79 %) in batch AD reactors. The HWE hydrolysates from pretreatment temperature of 170 °C gave a 13 % lower methane yield for birch compared to hydrolysates pretreated at 140 °C (not significant decrease for Norway spruce) in batch AD, while it was 9 % lower for Norway spruce in continuous AD compared to hydrolysates pretreated at 140 °C. This is due to higher concentration of inhibitors (furans and soluble lignin) and possible extraction and formation of higher concentration of recalcitrant compound (soluble lignin) at higher temperature. Birch (hardwood) hydrolysate pretreated at 140 °C resulted in higher methane yield (8 %) than Norway spruce (softwood) as hemicellulose extraction is better in hardwood.

Hydrolysate of Norway spruce pretreated at 140 °C gave higher methane yield and improved production rate during mesophilic AD (35 °C) compared to thermophilic AD (55 °C) as thermophilic mixed cultures are more susceptible and sensitive to furan inhibitors. However, the result of hydrolysate pretreated at 170 °C was not consistent despite having higher concentration of furan inhibitors. Methane yield of hydrolysate pretreated at 170 °C decreased with increase in OL during the mesophilic AD while hydrolysate pretreated at 140 °C had similar methane yield at all OLs suggesting better performance of hydrolysate pretreated at 140 °C during higher OLs due to lower concentration of inhibitors compared to hydrolysate pretreated at 170 °C. During thermophilic condition, both hydrolysates pretreated at 140 °C and 170 °C were affected negatively with increasing OLs.

APL of birch from pyrolysis temperature at 400 °C and 550 °C had a methane yield of 44 % and 49 %, respectively, while a large decrease in methane yield from 59 % to 32 % was observed from the APL of Norway spruce with the increase in pyrolysis temperature from 400 °C to 550 °C, respectively, suggesting that increase in pyrolysis temperature might have increased the concentration of phenols in APL of softwood compared to hardwood as softwood has a higher concentration of lignin, which resulted in lower methane yield. Methane yield from APL of hot water extracted birch at 140 °C and 170

°C before pyrolysis (400 °C) improved compared to APL from non-hot water extracted birch and can be attributed to the removal of inhibitors while increasing sugar concentration during HWE. However, HWE at 140 °C before pyrolysis gave lower methane yield from Norway spruce APL had inconsistent result while HWE at 170 °C had no significant effect.

A co-digestion ratio of 3:1 (Hydrolysate:APL) improved the methane yield by 40 % and 6 % in Norway spruce and 26 % and 59 % in birch pretreated at 140 °C and 170 °C, respectively, compared to the 1:1 ratio suggesting that adding APL only as an additive is beneficial in terms of methane yield, rate and digestion time than considering as sole AD feed.

Keywords: Anaerobic digestion (AD), Lignocellulosic woody biomass, Hot water extraction (HWE), Hydrolysate, Aqueous pyrolysis liquid (APL), Co-digestion

List of articles

Article 1

Ghimire, N., Bakke, R., Bergland, W.H. (2020) Thermophilic Methane Production from Hydrothermally Pretreated Norway Spruce (Picea abies). Applied Sciences. 10, 4989. doi: 10.3390/app10144989.

Article 2

Ghimire, N., Bakke, R., Bergland, W.H. (2020) Mesophilic Anaerobic Digestion of Hydrothermally Pretreated Lignocellulosic Biomass (Norway Spruce (*Picea abies*)). Processes. 9(2), 190. doi: 10.3390/pr9020190

Article 3

van der Wijst, C., **Ghimire, N**., Bergland, W.H., Toven, K., Bakke, R., Eriksen, Ø. (2020) Improving Carbon Product Yields in Biocarbon Production by combining Pyrolysis and Anaerobic Digestion. Submitted to the Journal: BioResources (under review)

Article 4

Ghimire, N., van der Wijst, C., Toven, K., Eriksen, Ø., Bakke, R., Bergland, W.H. (2020) Methane Production in Cascade Processing of Woody Biomass. Submitted to Journal of Cleaner Production

Article 5

Ghimire, N., Bakke, R., Bergland, W.H. (2020) Liquefaction of Lignocellulosic Biomass for Methane Production: A Review. Submitted to Bioresource Technology

Other contributions

van der Wijst, C., **Ghimire, N**., Bergland, W.H., Toven, K., Bakke, R., Eriksen, Ø. (2020) Improved Carbon Balance for Biochar Production by Combination of Hot Water Extraction, Pyrolysis and Anaerobic Digestion. Manuscript in preparation.

Karlsen, V.B., **Ghimire, N.**, Bakke, R., Bergland, W.H. (2020) Anaerobic Digestion of Hemicellulose Sugars Implemented in ADM1. Proceedings of the 61st Conference on Simulation and Modeling (SIMS 61).

Kenarsari, Z.N., **Ghimire, N.**, Bakke, R., Bergland, W.H. (2020) Thermophilic Anaerobic Digestion Modeling of Lignocellulosic Hot Water Extract using ADM1. Proceedings of the 60th Conference on Simulation and Modeling (SIMS 60), 170, 125-131, 2020. doi: 10.3384/ecp20170125.

Aryal, N., **Ghimire, N**., Bajracharya, S. (2020) Coupling of Microbial Electrosynthesis with an Anaerobic Digestion for Waste Valorization, Editor (s): Li, Y., Khanal, S. K. Advances in Bioenergy. doi: 10.1016/bs.aibe.2020.04.003.

List of tables

Table 1.1: Effects of HWE and steam pretreatment on lignocellulosic biomass (adapted from (Antunes et al., 2019; Hendriks and Zeeman, 2009; Seidl and Goulart, 2016; Sun et al., 2016; Zheng et al., 2014; Zhuang et al., 2016)). 8

Table 1.2: Different modes of pyrolysis technologies, corresponding process conditions and typical product weight yield 11

Table 3.1 Severity factor for the hydrolysates from HWE 27

List of figures

Figure 1.1: Flow chart showing cascade processing of Norway spruce and birch producing biogas, bio-oil (APL and organic phase) and biochar. 3

Figure 1.2: Schematic biochemical pathways of AD (Adapted from (Batstone et al., 2002)). 4

Figure 1.3: Structural composition of lignocellulosic biomass (Adapted from (Alonso et al., 2012)). 5

Figure 1.4: Flow chart of APL production from lignocellulosic biomass after pyrolysis. 10

Figure 3.1: Sketch of the pyrolysis rig at RISE PFI. 28

Figure 3.2: AMPTS II set-up and equipment description (figure from Bioprocess control's homepage). 29

Figure 3.3: Syringe batch reactor test set-up with 100 mL medical syringe. 30

Figure 3.4 A) Sketch of lab-scale AD reactor with influent and effluent pipes and separator. B) Diagram of schematic process line of lab-scale UASB. 31

Figure 3.5: Integration of APL from pyrolysis of hot water extracted lignocellulosic biomass and AD. 33

Figure 4.1 Methane yields of Norway spruce and birch hydrolysates, pretreated at 140 °C and 170 °C, under mesophilic and thermophilic conditions at organic load of 10 and 20 g COD/L in AMPTS II. 36

Figure 4.2 Variations of methane yield of Norway spruce hydrolysates, pretreated at 140 °C and 170 °C, during mesophilic and thermophilic conditions at organic loadings of 6, 10, 20 and 30 g COD/L. 38

Figure 4.3 Methane yield of hydrolysates of Norway spruce pretreated at 140 °C and 170 °C under mesophilic and thermophilic AD conditions. 39

Figure 4.4 Methane production rates of hydrolysates of Norway spruce pretreated at 140 °C and 170 °C under mesophilic and thermophilic AD conditions. 40

Figure 4.5 Biogas production during OLR increase over time during AD of Norway spruce hydrolysate pretreated at 140 °C. 42

Figure 4.6 Biogas production during OLR increase over time during AD of Norway spruce hydrolysate pretreated at 170 °C. 43

Figure 4.7 Methane yield of APL from pyrolysis of Norway spruce and birch at 400 °C and 550 °C. 45

Figure 4.8 Methane yield decreasing with increasing carbon content in APL. 45

Figure 4.9 Methane yield of APL from pyrolysis of Norway spruce at 400 °C with (at 140 °C and 170 °C) and without HWE. 47

Figure 4.10 Methane yield of APL from pyrolysis of birch at 400 °C with (at 140 °C and 170 °C) and without HWE. 48

Figure 4.11 Variation of methane yield with amount of APL during co-digestion with hydrolysate. 49

Abbreviations

AD – Anaerobic Digestion

AMPTS II – Automatic Methane Potential Test System

APHA – American Public Health Association

APL – Aqueous Pyrolysis Liquid

C/N – Carbon to Nitrogen ratio

CH_4 – Methane

CO_2 – Carbon dioxide

COD – Chemical Oxygen Demand

ESP – Electrostatic Precipitator

H_2 – Hydrogen

H_2S – Hydrogen Sulphide

HMF- Hydroxymethylfurfural

HP – Hewlett Packard

HPLC – High-performance liquid chromatography

HWE – Hot Water Extraction

L-AD – Liquid Anaerobic Digestion

LHW – Liquid Hot Water

MMLD – Mini Mill Laboratory Digester

N_2 – Nitrogen

NH_3 – Ammonia

OL – Organic Load

OLR – Organic Loading Rate

Py – Pyrolysis

RPM – Revolutions Per Minutes

SS-AD – Solid State Anaerobic Digestion

TAN – Total Ammonia Nitrogen

TS – Total Solids

UASB – Up flow Anaerobic Sludge Blanket

VFA – Volatile Fatty Acid

wt – weight

Table of contents

PREFACE I

ACKNOWLEDGEMENTS I

ABSTRACT III

LIST OF ARTICLES VI

LIST OF TABLES VIII

LIST OF FIGURES IX

ABBREVIATIONS XI

1 INTRODUCTION 1

1.1 Background 1

1.2 Anaerobic digestion 3

1.3 Composition of lignocellulosic biomass 5

1.4 Hot water extraction 7

1.5 Pyrolysis 9

1.6 Objectives 11

1.7 Approaches 12

1.8 Scope of the dissertation 13

2 LITERATURE REVIEW 14

2.1 Lignocellulosic biomass as feed for anaerobic digestion 14

2.2 Constraints and challenges for anaerobic digestion of lignocellulosic biomass 14

2.3 Anaerobic Digestion of hot water extract (hydrolysate) 16

2.4 Anaerobic Digestion of Aqueous Pyrolysis Liquid 19

2.5 Pretreatment inhibitors during anaerobic digestion 20

2.5.1 Sugar derivatives 21

2.5.2 Soluble lignin 22

2.5.3 Lignin derivatives 22

2.5.4 Inhibitory and toxic compounds from pyrolysis 23

2.6 Pretreatment tuning to increase overall anaerobic digestion yield 23

2.6.1 Hot water extraction 24

2.6.2 Pyrolysis 24
3 MATERIALS AND METHODS 26
3.1 Feed Preparation 26
3.1.1 Raw materials 26
3.1.2 Hot water extraction 26
3.1.3 Synthetic hydrolysate 27
3.1.4 Pyrolysis 27
3.2 Anaerobic digestion 28
3.2.1 Batch reactors 28
3.2.2 Continuous fed reactor (Unpublished) 30
3.2.3 Integration of hot water extraction and anaerobic digestion 32
3.2.4 Integration of pyrolysis and anaerobic digestion 32
3.2.5 Integration of hot water extraction, pyrolysis and anaerobic digestion 33
3.3 Analytical methods 34
3.3.1 Hot water extraction 34
3.3.2 Pyrolysis 34
3.3.3 Anaerobic digestion 34
4 RESULTS AND DISCUSSIONS 35
4.1 Anaerobic digestion of hydrolysate (Article 1, 2, 4 and unpublished results) 35
4.1.1 Effect of pretreatment severity and biomass types on methane yield and rate from Norway spruce and birch hydrolysate 35
4.1.2 Effect of organic load during anaerobic digestion of Norway spruce hydrolysate 37
4.1.3 Effect of anaerobic digestion temperature of Norway spruce hydrolysate 39
4.1.4 Continuous AD of Norway spruce hydrolysates in a high rate reactor (Unpublished results) 41
4.2 Anaerobic digestion of aqueous pyrolysis liquid (Article 3) 44
4.3 Anaerobic digestion of aqueous pyrolysis liquid from pyrolysis of hot water extracted biomass (Article 3 and 4) 46

4.3.1 Norway spruce 46
4.3.2 Birch 47
4.4 Anaerobic co-digestion of hydrolysate and APL 48
5 CONCLUSIONS 50
5.1 Effect of HWE pretreatment severity and biomass types on anaerobic digestion 50
5.2 Effect of organic load and temperature during anaerobic digestion of Norway spruce hydrolysate 51
5.3 Effect of pyrolysis temperature and biomass types on anaerobic digestion of aqueous pyrolysis liquid 51
5.4 Effect of hot water extraction before pyrolysis on anaerobic digestion of aqueous pyrolysis liquid 52
5.5 Anaerobic co-digestion of hydrolysate and aqueous pyrolysis liquid 53
5.6 Further recommendations 53
5.6.1 Anaerobic digestion process 53
5.6.2 Pretreatment of anaerobic digestion feed 54
5.6.3 Microbiology 54
5.6.4 Anaerobic digestion model 1 (ADM1) modeling 54
REFERENCES 55

Part I

1 Introduction

The potential of bio-methane is introduced in this chapter for enhanced energy recovery from by-product streams generated during the production of bio-carbon material from lignocellulosic biomass (woody biomass) which is used as a reducing agent and energy source paving a way for huge carbon dioxide (CO_2) emission reduction from metallurgic industries. Anaerobic digestion (AD), lignocellulosic biomass, hot water extraction (HWE) and pyrolysis are also introduced in this chapter which ends by setting objectives and scopes of the thesis and approaches taken to meet the objectives.

1.1 Background

Fossil fuel is still providing more than 80 % of the world energy demand and efforts should be made in providing new and renewable alternatives for energy security and to avoid environmental and health hazards caused by excessive dependence on fossil fuels (Guo et al., 2015). Metallurgic industries consume huge amount of carbon material as a reducing agent and energy source and replacing fossil carbon with bio-carbon (biochar) by exploiting the cheap and abundant woody biomass lignocellulosic biomass would lead to huge reduction in global CO_2 reduction. Traditional kilns without off gas utilization or recovery still produce majority of today's charcoal (Bailis et al., 2013) and should be replaced as they are emitting incomplete combustion products with larger global warming impact than the molar CO_2 equivalent of complete combustion of the off gases into the atmosphere (Bailis, 2009) and increasing mortality and respiratory diseases for populations close by the points of emission (Bailis et al., 2005).

Pyrolysis is a modern technology fundamentally similar to charcoal production in which thermal degradation of biomass takes place in oxygen free environment. Out of different types, intermediate pyrolysis has been in use in recent years with emphasis on co-production of bio-oil and biochar (Laird et al., 2009) as numerous applications and enormous environmental benefits of biochar have been recognized (Cha et al., 2016). It produces 30 wt. % biochar which is in the upper range of traditional charcoal kiln. Bio-oil, on the other hand, usually phase separates into an organic phase and aqueous phase (aqueous pyrolysis liquid, APL) out of which APL has no obvious area of applications despite being organic rich rendering it as a by-product waste stream. However, APL contains several compounds, both toxic and with unknown effects, making it a challenging feed for biological treatment (Kan et al., 2017).

Various pre-treatments can be applied on lignocellulosic biomass before pyrolysis to obtain biochar of better quality. HWE is one of such pre-treatments which is simple, cost-effective and environment friendly and removes hemicellulose significantly with a small part of lignin resulting in a solid residue with a higher content of lignin and cellulose, beneficial for biochar and bio-oil production (Nitsos et al., 2013). Hydrolysate, thus produced liquid stream, is organic rich but has no obvious use due to presence of inhibitors rendering it also as a by-product waste stream.

Both of the organic rich by-product streams, hydrolysate and APL, need to be well treated before disposing to avoid environmental deteriorations and enhance energy recovery. AD can be considered to handle these waste streams due to the presence of mixed communities of organisms capable of coping complex, toxic and inhibitory compounds (Benjamin et al., 1984). Moreover, AD produces biogas, a renewable and clean biofuel and improves energy recovery in the overall cascade processing of lignocellulosic biomass (Figure 1.1).

Lignocellulosic biomass types and operating conditions of both HWE and pyrolysis (temperature and retention time) play an important role in the quality of final product, either the main product biochar or by-product waste streams for efficient methane production from AD (both APL and hydrolysate). It is also imperative to choose the best possible AD operating conditions for efficient methane production.

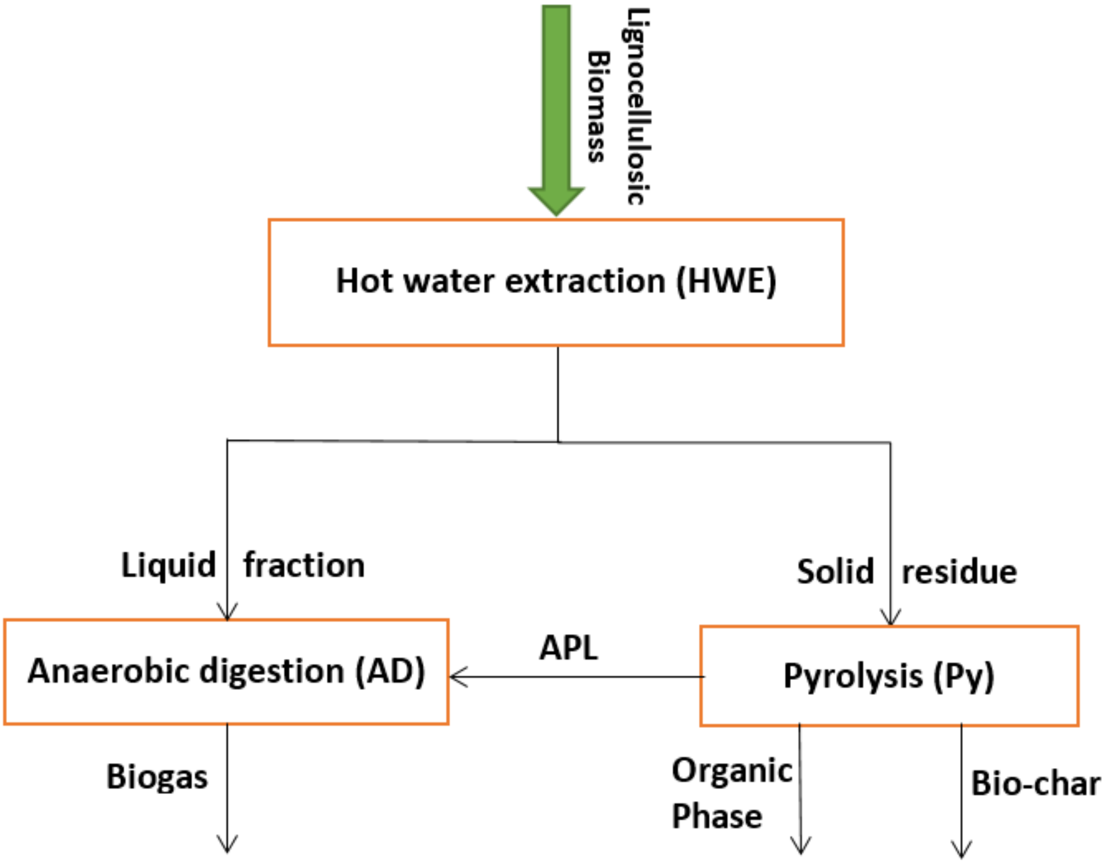

Figure 1.1: Flow chart showing cascade processing of Norway spruce and birch producing biogas, bio-oil (APL and organic phase) and biochar.

1.2 Anaerobic digestion

AD is a robust and mature biological process where a mixed community of microorganisms act together to break down organic compounds to produce biogas (about 50-75 % methane (CH_4), 25-50 % CO_2 and traces of hydrogen sulfide (H_2S), ammonia (NH_3), nitrogen (N_2), hydrogen (H_2), water vapor, siloxane other volatile compounds) in the absence of free oxygen) (Aryal and Kvist, 2018). An advantage of such consortia of microorganisms is their ability to synergistically break down various complex, recalcitrant and inhibiting compounds (in low concentration) to methane after some adaptation time (Benjamin et al., 1984). The AD process is carried out in four main steps, namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Figure 1.2). Initially, the complex organic substrate is converted to smaller compounds (e.g. volatile fatty acids (VFAs) and alcohols) during hydrolysis and acidogenesis where hydrolysis is the rate limiting step for complex substrate (Passos et al., 2017). VFAs and alcohols are further broken down to acetate, hydrogen and carbon dioxide during acetogenesis and finally to methane and carbon dioxide from the intermediates during methanogenesis and can be a rate limiting step for readily biodegradable substrates. Methanogenesis is carried out by archaea via two pathways viz. aceticlastic methanogenesis, carried out by heterotrophic organisms using acetic acid to produce CH_4 and CO_2 and hydrogenotrophic methanogenesis, carried out by autotrophic organisms using CO_2 and H_2 to produce CH_4. However, disruption in the symbiosis of different microorganisms leads to VFA accumulation due to stressful conditions such as nutrient deficiency and toxicity in the feed which may lead to poor biogas production and methane content and ultimately failure of the reactor (Baeta et al., 2016). Therefore, AD requires regular monitoring with proper process control for the optimization (Aryal et al., 2020).

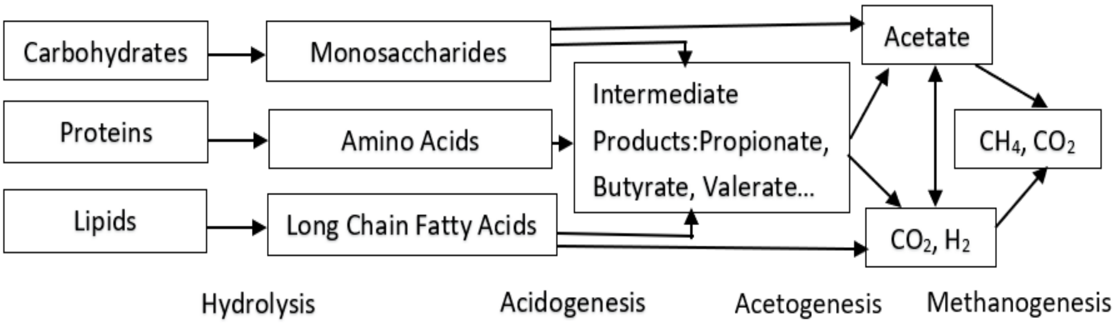

Figure 1.2: Schematic biochemical pathways of AD (Adapted from (Batstone et al., 2002)).

Temperature plays a crucial role in AD as increased temperature leads to increased reaction rate in biochemical systems (Li et al., 2015). Thermophilic AD (55-60 °C) is considered a highly-efficient system due to a better pathogen inactivation and enhanced biogas production rate compared to mesophilic AD (35-40 °C) (Li et al., 2015). However, several studies have reported that thermophilic AD is susceptible to process imbalance due to VFAs accumulation (especially propionic acid), higher risk of ammonia inhibition, and decreasing the pH-buffer system (Dinsdale et al., 1997; Lier et al., 1993; Nges and Liu, 2010). Thermophilic AD is opted if the feed preparation is operated at higher temperature which avoids the energy cost for heating the reactors. Besides the operating condition, higher proportion of feeding can also influence the rate of AD. The OL of the reactor with reasonable amount of inoculum is an important parameter when estimating methane potential (Hashimoto, 1989). High OL leads to VFA accumulation inhibiting the methanogens, thus lowering the amount of methane produced. On the contrary, lower OL cannot provide enough nutrition for microorganism growth, thus hampering the AD process (Feng et al., 2013).

1.3 Composition of lignocellulosic biomass

The composition of lignocellulosic biomass creates constraints and challenges for direct AD of the lignocellulosic biomass. Lignocellulosic materials are primarily composed of three types of polymers: cellulose $(C_6H_{10}O_5)_n$ (30-50 %), hemicellulose $(C_5H_8O_4)_m$ (15-30 %) and lignin $[C_9H_{10}O_3(OCH_3)_{0,9-1,7}]_x$ (10-20 %) (Akhtar et al., 2016; Jørgensen et al., 2007). Cellulose and hemicellulose are chain polysaccharides, while lignin, closely associated with cellulose and hemicellulose, is a heterogeneous, phenolic polymer (Mussatto et al., 2008) (Figure 1.3). The composition of lignocellulosic biomass depends on the plant species, its growth stage and environment (Surendra and Khanal, 2015). Non-structural carbohydrates like glucose, fructose, and sucrose along with proteins, lipids, and pectin are also present in different concentrations in the lignocellulosic materials (McDonald et al., 1991).

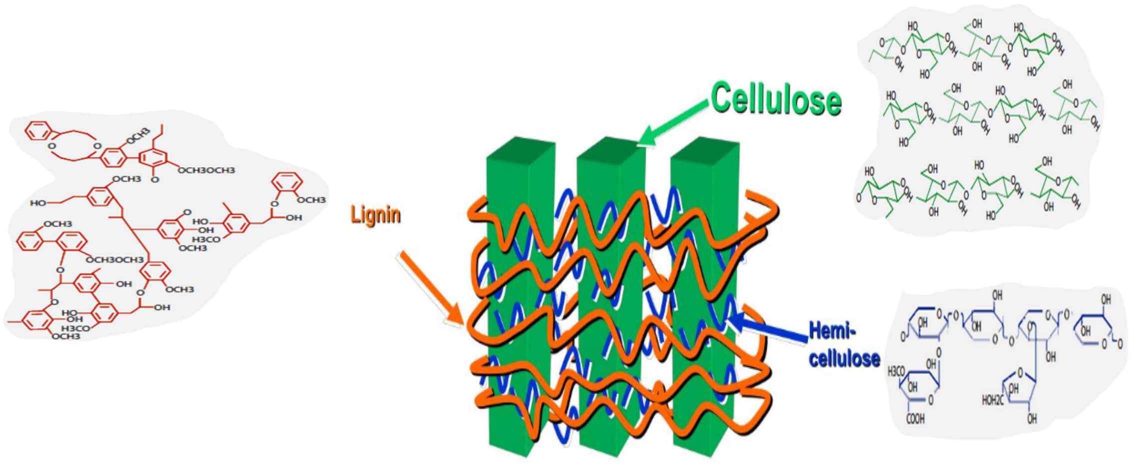

Figure 1.3: Structural composition of lignocellulosic biomass (Adapted from (Alonso et al., 2012)).

1.3.1.1 Cellulose

Cellulose is the main component of lignocellulose cell walls, produced biosynthetically and is the most abundant organic compound on earth (Zheng et al., 2014). It is a linear condensation polymer consisting of anhydro-glucan joined together by β,1-4 glycosidic bonds (Zhang and Lynd, 2004). These bonds give significant hydrogen bonding, in both intra- and inter-molecular cellulose molecules (Saini et al., 2015). The hydrogen bonds between the glucan units determines the crystallinity of cellulose, indicated by the crystallinity index (Zheng et al., 2014). Different orientation of cellulose material throughout the structure leads to different levels of crystallinity (Zheng et al., 2014) with low crystallinity classified as amorphous and high crystallinity as crystalline (Atalla and Vanderhart, 1984).

1.3.1.2 Hemicellulose

Hemicellulose has a complex carbohydrate structure more related to cellulose than lignin (Rydholm, 1965), and its content differ significantly from hardwood (angiosperm) to softwood (gymnosperm) (Fengel and Wegener, 1989). It is composed of pentoses (xylose, rhamnose and arabinose) and/or hexoses (glucose, mannose and galactose) and acids (glucuronic acid, methyl glucuronic acid, and galacturonic acid) (Zheng et al., 2014). In general, xylose is present at higher concentration as hemicellulose component in agricultural plants, such as grasses, straws and hardwoods (Kambo, 2014; Pu et al., 2008) while glucose and mannose are present at higher concentrations in softwood (Ebringerova and Heinze, 2000).

1.3.1.3 Lignin

Lignin is a cross-linked three-dimensional phenolic polymer generally combined with hemicelluloses (Fengel and Wegener, 1989). It is the most abundant aromatic biopolymer on earth (Righi et al., 2016), more concentrated in softwood than hardwood, and second most abundant organic compound after cellulose (Zheng et al., 2014). Lignin plays the role of cement in cross-linking cellulose and hemicellulose to form a rigid three-dimensional structure of the cell wall (Palmqvist and Hahn-Haagerdal, 2000). It protects plants from physical attack and gives structural strength (Paul and Dutta, 2018). P-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) are the basic units of lignin polymers (Xu and Ferdosian, 2017). Lignin is water insoluble (Zheng et al., 2014), but water at higher temperature (180 °C) can dissolve lignin depending upon how lignin is formed (Grabber, 2005).

1.4 Hot water extraction

Hot water extraction (HWE, also called liquid hot water (LHW) or hydrothermolysis) is a mild hydrothermal pretreatment carried out in the temperature range 120-230 °C and at various pressure conditions at which water is kept in subcritical conditions (Nitsos et al., 2013). Its objective is to efficiently extract sugars, acids, or other chemicals from lignocellulosic biomass without considering any structural changes in the extracted wood (Pelaez-Samaniego et al., 2013). During HWE, water or steam (or both) penetrates under pressure (with or without catalysts) to liberate most of the hemicellulose and partial lignin while making biomass more accessible to hydrolytic enzymes (Pérez et al., 2007; Taherzadeh and Karimi, 2008). The composition of aqueous hemicellulose depends on the sources of lignocellulosic biomass and the extraction time and temperature. At pretreatment temperature of 100 °C, hemicellulose remains in the solid fraction but at temperature above 150 °C, hemicellulose hydrolyzes and dissolves into the liquid fraction (or hydrolysate) (Fernandez-Cegri et al., 2012; Hendriks and Zeeman, 2009). At temperature 150-180 °C, solubilization of firstly hemicellulose and shortly after lignin starts (Bobleter, 1994; Garrote et al., 1999; Hendriks and Zeeman, 2009). HWE has different effects on different components of lignocellulosic biomass (Table 1.1).

Table 1.1: Effects of HWE and steam pretreatment on lignocellulosic biomass (adapted from (Antunes et al., 2019; Hendriks and Zeeman, 2009; Seidl and Goulart, 2016; Sun et al., 2016; Zheng et al., 2014; Zhuang et al., 2016)).

Components	Effects on the composition and structure	Effects of HWE
Cellulose	Increase contact surface area	High
	Reduce particle size	Inefficient
	Reduce degree of polymerization	Partial
	Reduce crystallinity	Partial
	Increase solubilization	Inefficient or low
Hemicellulose	Increase solubilization	High
	Formation of degradation products (e.g furfural, HMF)	Partial
Lignin	Structural change	Partial
	Increase solubilization	Inefficient or low
	Formation of degradation products (e.g, phenol)	Inefficient or low

HWE can be carried out for conditioning woody biomass to optimize production of other products such as biochar, bio-oil and valuable products (Alvarez-Chavez et al., 2019; Pelaez-Samaniego et al., 2013; Rasi et al., 2019). Hot water extract (hydrolysate) is produced as a by-product side stream during the HWE pretreatment of woody lignocellulosic biomass to enhance the quality of the solid biomass products like composites and various board products, such as reduced water absorption, improved mechanical properties and improved resistance to decay (Pelaez-Samaniego et al., 2013); improve bio-oil yield due to increased cellulose content while reducing ketones, acids and water content in the bio-oil leading to higher heating value and significantly improved levoglucosan content (Chang et al., 2013); and lower the activation energy for thermal degradation during pyrolysis compared to untreated biomass (Kumar et al., 2020).

1.5 Pyrolysis

Pyrolysis breaks down chemical bonds to form new compounds in the absence of oxygen and has a high flexibility in processing raw biomass materials for derived end products (Rasi et al., 2019). It converts biomass thermo-chemically into liquid, charcoal and non-condensable gases (syngas) by heating to about 480 °C or more (Demirbas, 2000). Higher cellulose content leads to higher liquid products, high hemicellulose content leads to higher gas production and higher lignin content leads to more solid residues (Kumar et al., 2020).

The liquid which is usually called bio oil is also called pyrolysis oil, pyrolysis liquid, bio-crude, wood liquid, wood oil or wood distillate (Mohan et al., 2006) and consists of molecules derived from the degradation of cellulose, hemicellulose and lignin (Alvarez-Chavez et al., 2019). Bio oil from pyrolysis is a dark brown, free flowing liquor with pungent smell that consists of a complex mixture of up to 400 organic compounds such as acids, sugars, alkenes, esters, ethers and different oxygenates (Evans and Milne, 1986; Huber et al., 2006; Kan et al., 2017; Rezaei et al., 2014). Bio oil can be upgraded to fuels due to the presence of phenolic monomers and dimers (Mortensen et al., 2011), its ideal carbon numbers (C_6-C_{20}) and relatively lower oxygen content compared to carbohydrate (Zhou et al., 2019). Similarly, engineered microorganisms (e.g. *Escherichia coli*) can utilize the pyrolytic sugars such as levoglucosan to produce fuels and chemicals (Layton et al., 2011).

However, high water content of bio oil poses several challenges such as low heating value and chemical and thermal instability (Zhou et al., 2019). Due to the presence of high concentration of water in the feedstocks, the bio oil is separated into aqueous phase (APL) (lighter fraction) and organic phase (heavy fraction) (Figure 1.4). The organic phase (or biocrude) is a complex mixture of oxygenated hydrocarbons and nitrogenated compounds such as aromatics, short chain carboxylic acids, ketones, phenolics, sugars and derivatives of furan depending upon the type of biomass (Hassan el et al., 2009). Its complex nature together with high oxygen levels makes it difficult to utilize this organic phase directly in AD and can enter the market as renewable alternative to heavy fuel oil (Oasmaa et al., 2015). The organic or oily phase is considered as an energy source and for chemical production (Rasi et al., 2019), and it has also been considered used for bio-based pesticides (Hagner et al., 2018) and in manure acidification (Keskinen et al., 2017).

APL, on the other hand, has high chemical oxygen demand (COD) concentration along with various potentially toxic organic compounds and can be environmentally harmful if not managed properly (Seyedi et al., 2019). APL has a high water content and contains C_2-C_6 sugars, furan derivatives, hydroxyacids, oligomers, water soluble phenols and other water soluble organics formed during pyrolysis (Shanmugam et al., 2017).

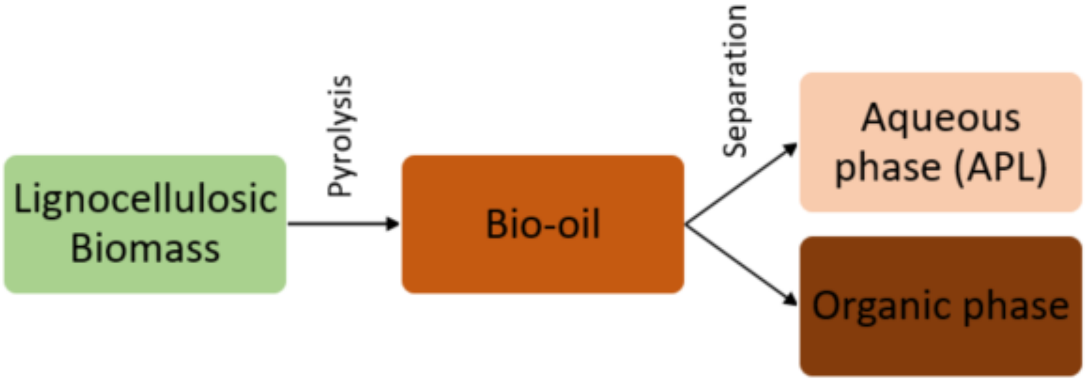

Figure 1.4: Flow chart of APL production from lignocellulosic biomass after pyrolysis.

Process parameter adjustments varies the proportion of produced bio oil, biochar and syngas. Lower process temperature and longer vapor residence time favors char production. High temperature and longer residence time increases syngas formation, while moderate temperature and short vapor residence time produces optimum bio oil (Bridgwater, 2012). Pyrolysis is differentiated into four main categories based on residence time, slow (or conventional), moderate (or intermediate), fast and flash (Table 1.2) (Bridgwater, 2012; Giwa et al., 2019; Goyal et al., 2008; Sharma et al., 2015). In fast pyrolysis, liquid with only a single phase is obtained (Oasmaa et al., 2015) while intermediate pyrolysis produces two phase liquid (organic phase and aqueous phase (APL)) (Fabbri and Torri, 2016).

Table 1.2: Different modes of pyrolysis technologies, corresponding process conditions and typical product weight yield

Mode	Conditions		Products		
	Temperature (°C)	Residence time	Liquid (%)	Char (%)	Gas (%)
Fast	~500	~1 s	75	12	13
Moderate	~500	~10-30 s	50	20-25	25-30
Slow	~500	~5-30 min	30	35	35
Flash	400-950	30 ms-1.5 s	70	25	16

1.6 Objectives

The main task of this PhD work is to consider the by-product streams which are produced during the production of biochar, a renewable source of carbon material from lignocellulosic biomass used as a reducing agent and energy source, as feed for AD to produce methane, a renewable energy, to increase the overall energy recovery.

During the process of biochar production by intermediate pyrolysis, organic rich but complex APL is produced. Similarly, hemicellulosic sugars rich hydrolysate is produced when biomass is pretreated by HWE. Both APL and hydrolysate are waste streams and considered as AD feed for enhanced energy recovery before discarding to avoid environmental deteriorations.

This PhD work aims to contribute to this effort of enhancing energy recovery by improved utilization of lignocellulosic biomass. The main objectives of this PhD work are:

- Study the effects of the biomass types (birch (hardwood) and Norway spruce (softwood)) and pretreatment severity (temperature (170 °C and 140 °C) and retention time (300 min and 90 min)) on lignocellulosic biomass during HWE on kinetics and methane yield from the hydrolysate in batch reactors. Study hydrolysate of Norway spruce as a model substrate at mesophilic continuous AD condition.
- Comparative study of the effects of AD temperature conditions (mesophilic (35 °C) and thermophilic (55 °C)) and OL on kinetics and methane yield from the hydrolysate of Norway spruce (softwood) as a model substrate.
- Study the effects of pyrolysis temperature (400 °C and 550 °C) and biomass types (birch (hardwood) and Norway spruce (softwood)) on kinetics and methane yield from the APL. Also, to study the effect of HWE (170 °C and 140 °C) before pyrolysis (400 °C) on methane yield from the APL.
- Evaluate co-digestion of hydrolysate and APL from birch and Norway spruce for enhanced methane production.

1.7 Approaches

Various approaches have been used in order to meet the objectives such as:

- Use of automatic biomethane potential test system (AMPTS II) and syringe as batch reactors to obtain kinetic and stoichiometric data by methane potential tests.

- Use up-flow anaerobic sludge bed (UASB) reactor tests to investigate high rate AD of the hydrolysates, including adaptations to inhibitors.
- Use the available range of analysing techniques available for feed, bioreactor environment and effluent characterization.

1.8 Scope of the dissertation

The scopes of the PhD work are as follows:

- Literature study of:
    - AD of lignocellulosic substances, including HWE and pyrolysis of lignocellulosic biomass and inhibition from degradation products.
    - HWE method for efficient hemicellulose extraction for use as feed for AD.
    - APL for use as feed for AD.
- Conduct batch experiments using AMPTS II and syringe reactors.
- Build, operate and investigate process performance of UASB reactor with continuous feed.

2 Literature review

Review of different scientific works related to suitability of lignocellulosic biomass as feed for AD, its constraint and promising AD results of hydrolysate and APL is discussed in this chapter.

2.1 Lignocellulosic biomass as feed for anaerobic digestion

Large amount of cellulose and hemicellulose makes lignocellulosic biomass attractive for biogas production through AD (He et al., 2015). Lignocellulosic biomass has competitive advantages over other AD feedstock due to their abundance, low price, relatively consistent composition and relatively high yield (Wu and He, 2013). It can however vary to what extent these feedstocks can be treated in sustainable ways by AD to produce biogas. A wide range of lignocellulosic biomass has been considered as feeds for AD (Gunaseelan, 1997) including agricultural and forestry residues, municipal paper and food waste (Sawatdeenarunat et al., 2015; Zeng et al., 2007); waste lignocellulosic substances like wood and wood wastes (Nakamura and Mtui, 2003); agricultural crops and their waste residues like sugarcane bagasse (Baeta et al., 2016), corn silage, cob and stover (Huang et al., 2017; Li et al., 2013a; Li et al., 2014; Wirth and Mumme, 2014), wheat and rice straw (Chandra et al., 2012a; Chandra et al., 2012b; Kaparaju et al., 2009a; Kaparaju et al., 2009b; Nkemka and Murto, 2013); animal waste (Bergland et al., 2015); industrial residue such as paper and pulp processing waste (Ashrafi et al., 2015); energy crops such as giant reed and Napier grass (Di Girolamo et al., 2013; Wells et al., 2019); and municipal solid waste (Pecorini et al., 2016).

2.2 Constraints and challenges for anaerobic digestion of lignocellulosic biomass

Lignocellulosic biomass can be treated anaerobically to produce biogas. However, AD is not sufficient to recover all the energy from lignocellulosic materials because of a high content of non-biodegradable lignin and a high crystallinity of cellulose, and the lignocellulosic matrix limits the accessibility of the sugars, making pretreatments compulsory to exploit the potential of such substrates. Hydrolysis, the rate-limiting step for lignocellulosic material that breaks down the complex organic polymer components during AD, is a very slow process for lignocellulosic materials and is sensitive to the type and composition of the substrate (Paudel et al., 2017) where each component has its own degree of resilience to chemically induced, thermal and biological degradation (Liu, 2015). The hydrolysis is affected by the high crystallinity of cellulose limiting the surface available for biodegradability (Frigon and Guiot, 2010). A higher crystallinity of cellulose, indicated by the crystallinity index, means more difficulty in biodegradation (Zheng et al., 2014), where amorphous cellulose is more susceptible to microbial degradation than crystalline cellulose (Monlau et al., 2013). In addition, both the hemicellulose and the lignin fractions act as physical barriers to avoid enzymatic attack of cellulose (Hu and Ragauskas, 2012). Lignin is the main barrier for AD of lignocellulosic biomass, the higher the lignin content in the biomass; the more difficult it is to biodegrade. Lignin must be opened up and/or dissolved in a pretreatment process to make the lignocellulosic sugars available for efficient AD but is not a main source for methane production itself.

Other constrains to the AD process is the heterogeneity and low density of lignocellulosic substances that hamper AD by forming a floating layer on the surface of AD reactors (Tian et al., 2015). This leads to poor substrate accessibility for the microorganisms, worsening mass and heat transfer and reducing the methane yield (Wang et al., 2018). High carbon to nitrogen (C/N) ratio can also limit AD of lignocellulosic biomass (Sawatdeenarunat et al., 2015) but it can be adjusted by adding nitrogen sources (at added cost) or co-digesting with substrate with higher nitrogen content.

Reported AD of lignocellulosic biomass is mostly solid state AD (SS-AD) which is operated at a total solid (TS) content of 15 % or higher, contrary to liquid-state AD (L-AD) that operates at TS content of less than 15 % (Guendouz et al., 2010; Rapport et al., 2008). SS-AD has several advantages but also several inherent problems. Some of the well noted problems are low methane yield, slow methanogenesis, slow mass transfer between AD microorganisms and feedstock, potential instability, acidification, ammonia inhibition, nutrient imbalance, temperature disturbance and obstacles in using end products (Yang et al., 2015).

Pretreatments can efficiently deal with the slow hydrolysis of lignocellulosic materials by disrupting the lignin barrier, solubilizing the hemicellulose, reducing the cellulose crystallinity, increasing the surface for enzymatic attack and homogenizing the lignocellulosic biomass to improve biodegradability (Bhatia et al., 2020; Carrere et al., 2016; Di Girolamo et al., 2013; Sawatdeenarunat et al., 2015). Pretreatments can be categorized into different groups such as mechanical or physical, thermal, hydrothermal, chemical and biological. Pretreatments involving chemical tend to be costly both due to added costs of chemicals and handling of chemical waste streams and should be avoided if possible.

Pretreatment for liquefaction of lignocellulosic biomass overcomes problems posed by SS-AD. Approach of liquefying lignocellulosic biomass is the most disruptive approach as it changes the inlet mode of lignocellulosic biomass from solid to liquid so that a wider range of AD reactors, including high rate sludge bed, can be applied. Liquid state AD (L-AD) also has greater reaction intensity and shorter retention time (Kainthola et al., 2019). Hot water extract and APL are the liquid feed with soluble organics produced as by-product streams by HWE and pyrolysis, respectively, which can be used in AD process overcoming the problems posed by conventional SS-AD for enhanced methane production.

2.3 Anaerobic Digestion of hot water extract (hydrolysate)

Biomethane potential of hydrolysate cannot be predicted on the basis of HWE treatment alone due to factors such as difference in hemicellulose content between different lignocellulosic biomass, generation of inhibitors, hydrothermal reactor configuration, liquid and solid ratio, operation mode i.e. batch or continuous and possible utilization of catalytic agent (Ahmad et al., 2018).

The biomethane potential is however clearly influenced by HWE pretreatment temperature with some optimal combination of time and temperature determining the amount of sugars transferred to the liquid hydrolysate and the amounts of inhibitory compounds included. The choice of pretreatment severity (based on temperature and residence time) depends on the feedstock. Low severity pretreatment conditions form products such as oligosaccharides (xylo-oligosaccharides) of higher potential value due to their prospective use in medicinal, food, cosmetic and health products (Qing et al., 2013). Higher severity leads to better extraction of hemicellulose from the biomass but forms inhibitors from degradation of monosaccharides extracted, with possible appearance of metal ions from reactor vessel in the extracted hydrolysate (Carvalheiro et al., 2016).

Recent studies suggest that operating temperature between 100 and 230 °C is best to obtain hydrolysate for biogas production (He et al., 2015) and HWE pretreatment temperatures above 250 °C is not recommended to avoid unwanted pyrolysis reactions (Brownell et al., 1986). If biochar is the main product of the process, temperature should be chosen (dependent on the biomass type) to extract hemicellulose as much as possible while avoiding lignin and cellulose solubilization. Review by He et al. (2015) covers specific operational parameters crucial for lignocellulosic biomass for biogas production.

The products from HWE are results of different temperatures, pressures and water contents, forming a vast variety of components, from easily degradable to inhibiting. Hydrolysate consists mainly of oligomeric and monomeric hemicellulose sugars and is beneficial to the AD in theory (Mosier et al., 2005b). HWE should liberate compounds utilized by AD microorganisms such as pentose sugars (xylose and arabinose), hexose sugars (mannose, galactose, glucose), volatile fatty acids (VFA), proteins and lipids for effective AD process (Bruni et al., 2010). During HWE, part of the hemicellulose is hydrolyzed and form acids such as acetic acid which is the most abundant VFA produced while other VFAs are found to be negligible (Di Girolamo et al., 2013). The produced acetic acid, which can be directly converted methane production, also acts as a catalyst during the process in degrading the polymers and thereby contributes in increasing the sugar yield (Hu and Ragauskas, 2012; Mosier et al., 2005b; Xu et al., 2019).

However, moderate formation of inhibitory products will occur during HWE, hampering the methane yield (McMillan, 1994; Sun et al., 2016; Zheng et al., 2014). Increase in temperature during pretreatment leads to formation of AD inhibitors like furans from sugars degradation (Mosier et al., 2005a; Simangunsong et al., 2018) and phenolic compounds from lignin polymer and/or lignin oligomers such as vanillin and syringaldehyde (Barakat et al., 2012; Klinke et al., 2002; Monlau et al., 2014). The furan compound 2-furaldehyde, referred to as furfural, forms due to degradation of pentose while 5-hydroxymethyl-2-furaldehyde, referred to as 5-HMF, forms because of hexose degradation (Jonsson and Martin, 2016; Larsson et al., 1999). Less 5-HMF is formed compared to furfural due to limited hexose degradation (Chandel et al., 2011). These degradation products are inhibitory to bacterial fermentation and can lower the methane yield in AD (Palmqvist et al., 1999).

As part of lignin can be dissolved along with the hemicellulose (Garrote et al., 1999; Mosier et al., 2005b), it can inhibit different steps of AD (Koyama et al., 2017). Soluble lignin itself is either inert (Jimenez et al., 1990; Liu et al., 2017) or has very low biodegradability under anaerobic condition (Benner et al., 1984), but hemicellulosic sugars, which are readily biodegradable, appear to be less biodegradable or even completely refractory when in combination with lignin (Dauwe et al., 2007; Djajadi et al., 2018; Jimenez et al., 1990; Li et al., 2018; Tong et al., 1990). In addition, partial lignin degradation leads to inhibitory compounds.

Hydrolysate produced due to HWE as pretreatment of agricultural residues has been successfully used as AD feed (Baeta et al., 2016; Luo et al., 2019). The enhanced methane production due to HWE pretreatment for different agricultural residues, compared to untreated ones, is reported to be from 20 % for wheat straw (Chandra et al., 2012b) to 222 % for rice straw (Chandra et al., 2012a). Although SS-AD of *Eucalyptus globulus,* a woody biomass, has been examined in AD after HWE (Nakamura and Mtui, 2003), reported HWE of woody biomass is limited mostly to make woody biomass rich in cellulose and lignin by removing hemicellulose (Pelaez-Samaniego et al., 2013), not considering L-AD for methane production. This by-product stream can be handled by AD but in depth, evaluations of such solutions are lacking.

2.4 Anaerobic Digestion of Aqueous Pyrolysis Liquid

The products from pyrolysis are results of different temperatures and residence time, forming a vast variety of components, from easily degradable to inhibiting. APL contains soluble organic fractions such as sugars, acetic acid, methanol, furfural, formaldehyde, HMF and phenol and are considered as a feedstock for biogas production as these compounds can be converted to methane by AD microorganisms (Cordella et al., 2012). As AD consumes acetic acid directly to produce methane, it is a suitable technology to use this acetic acid rich substance (Wen et al., 2020).

However, APL from pyrolysis is a challenging feed as it consists of known inhibitory organic compounds such as cresol, hydroxyacetaldehyde, acetol, furans, N-heterocyclic compounds and phenols together with several compound with unknown effect (Evans and Milne, 1986; Fedorak and Hrudey, 1984; Huber et al., 2006; Kan et al., 2017; Liaw et al., 2015; Rezaei et al., 2014).

Pyrolysis in conjunction with AD (Py-AD) is gaining interest as a low cost environmental friendly option with some investigations already started (Cordella et al., 2012; Hubner and Mumme, 2015). Life cycle analysis assessment performed on the coupling of AD and pyrolysis has shown significant achievable reduction of greenhouse emission (Righi et al., 2016).

APL from woody biomass has been subjected to AD for methane production. APL from Douglas fir wood has been examined in AD and increased methane production was observed in acetic acid (10 %) washed biomass compared to untreated, due to increased concentration of levoglucosan and reduced concentration of hydroxyacetaldehyde (Liaw et al., 2020). Continuous AD of APL from pine wood in UASB showed that 52 % of APL was potentially biodegradable at organic loading rate (OLR) of 1.5 g COD/(L d) (Torri et al., 2020). This should trigger more research on adaptation to this challenging substrate in continuous flow AD to make it feasible for industrial scale.

AD of APL from pyrolysis has been tested both through co-digestion and as a sole substrate. Inhibition was targeted in batch tests, with observed efficient anaerobic degradation of APL up to 1 g/L and complete inhibition at 20 g/L initial APL content (Willner et al., 2004) suggesting dilution through co-digestion as a reasonable solution. Co-digestion of up to 6.5 % (v/v) pyrolignitic acids (similar to APL), from pyrolysis of wood residue, mixed with swine manure is reported for a biofilm based AD (Andreoni, 1990). Recently, APL has been used as an additive during the AD of swine manure and observed improved methanogenic capacity because of trace elements in APL and enhanced resistance of microorganisms to high total ammonia nitrogen (TAN) concentration and its accumulation (Yu et al., 2020). Optimized integration of pre- and post-treatments together with adapted mixed AD cultures may be the key for full-scale AD of APL (Fabbri and Torri, 2016).

Although, experiment on APL of hot water extracted pine and spruce bark was performed recently which showed promising result (Rasi et al., 2019), AD of APL from hot water extracted woody biomass is still rare. Also, AD of undiluted APL of woody biomass without co-digestion or using acclimatized inoculum has not been explored more.

2.5 Pretreatment inhibitors during anaerobic digestion

The most prominent AD inhibitors produced during HWE are furfural and HMF together with soluble lignin and its derivatives. A range of measures have been suggested to counteract inhibition problems (Jonsson and Martin, 2016; Kim, 2018; Zabed et al., 2019). Readers interested in details regarding detoxification of hydrolysates are suggested review articles by Jönsson et al. (2013), Bhatia et al. (2020), Ko et al. (2015) and Kumar et al. (2019).

The pyrolysis process also produces several toxic compounds to microorganism at percentage level but several studies have shown that AD microorganisms adapt to these compounds (Barakat et al., 2012; Benjamin et al., 1984; Fox and Noike, 2004). The ratio of inhibiting compounds to inoculum influences the adaptation process (Park et al., 2012). Powdered activated carbon (PAC) and biochar have also been found useful in mitigating inhibitory effects (Liu et al., 2017; Torri and Fabbri, 2014; Zhou et al., 2015). Although biochar cannot be degraded to produce methane (Mumme et al., 2014), it can be added during AD to help in detoxification of APL while also supporting biofilm, favoring bio-methanation or enhancing electron transfer (Chen et al., 2014; Conti et al., 2014; Shen et al., 2015b; Xu et al., 2015; Zhao et al., 2015) thereby enhancing the biogas production. Developing a robust microbial consortia tolerant to toxicity of APL through metabolic evolution of organism has also been found effective for successful AD (Zhou et al., 2019).

2.5.1 Sugar derivatives

Sugar can degrade to furanic compounds which at high concentrations hamper microorganisms by inhibiting cell growth, inducing DNA damage and inhibiting several enzymes of the glycolysis pathway (Almeida et al., 2009; Palmqvist and Hahn-Haagerdal, 2000). Complete inhibition on methanogenic activity from furfural and HMF was observed at the concentration of 2 g/L while partial inhibition was observed starting from the concentration of 0.8 g/L depending on substrate compositions and inoculum in batch, and the inhibition is more pronounced during thermophilic condition than mesophilic condition (Ghasimi et al., 2016). Furfural and HMF may have additive inhibitory effects when both are present (Taherzadeh et al., 1999), while furfural is found to be more inhibitory compared to HMF due to its lower molecular weight which eases its uptake by microbial cells (Modig et al., 2002; Quéméneur et al., 2012).

However, furfural and HMF has also been found to work as sole carbon sources (at ~2 g/L) to produce methane (Barakat et al., 2012). During AD both furfural and HMF can be converted to less inhibitory compounds such as furfuryl and HMF alcohols by facultative anaerobes in AD, while furfural can also be converted to furoic acid and acetate, before being converted to methane and carbon dioxide (Liu et al., 2004; Monlau et al., 2014; Rivard and Grohmann, 1991). For detailed mechanism of inhibitory products formation during hydrothermal pretreatment, readers are referred to Nitsos et al. (2013).

2.5.2 Soluble lignin

Soluble lignin dissolved along with the hemicellulose hinders efficient AD of hydrolysate by inhibiting the growth of methanogens (Baeta et al., 2016; Barakat et al., 2012), with negative linear correlation between lignin content and methane yield during AD of cellulose, hemicellulose, manure wastes and acetate-rich wastewater (Li et al., 2018; Li et al., 2013b). A reduced AD rate for methanogenesis, acidogenesis and hydrolysis by 15 %, 10 % and 35 %, respectively, at soluble lignin concentration higher than 5 g/L is observed (Koyama et al., 2017).

Negligible amounts of lignin is broken down during AD with only 2-7 % of methane produced from lignin during co-digestion of natural lignin (organosolv, kraft and lignosulfonates) with xylose (Barakat et al., 2012), and only 1.4 % biodegradation of hardwood lignin compared to 16.9 % of grass lignin (Benner et al., 1984). However, elevated AD temperature was found to enhance conversion rate of lignin and lignified substances to methane or lower molecular-weight aromatic compounds during AD (Benner and Hodson, 1985).

2.5.3 Lignin derivatives

Lignin derivatives such as soluble phenolic compounds are formed at pretreatment conditions above 160 °C (Hendriks and Zeeman, 2009). The produced phenolic compounds have in many cases inhibitory and toxic effects on bacteria and methanogens/archaea (Campos et al., 2009; Gossett et al., 1982; Hierholtzer et al., 2013) as they can damage cell membranes, causing leakage of intracellular components and lead to inactivation of essential enzymatic systems (Heipieper et al., 1994). Inhibition by lignin derived phenolic compounds is directly related to molecular weight; lower molecular weight are more toxic than high molecular weight phenolic compounds (Clark and Mackie, 1984).

Microorganisms are however capable of adapting to soluble phenolic compounds (Hendriks and Zeeman, 2009) with effective phenol degradation during AD (Fang et al., 2004; Olguin-Lora et al., 2003; Rosenkranz et al., 2013) and Benzoate as a key intermediate (Fang et al., 2004; Hoyos-Hernandez et al., 2013). AD efficiency of different phenols depends on temperature with higher degradation efficiency at mesophilic than at thermophilic conditions (Leven et al., 2012; Levén and Schnürer, 2005). Some degradation can also occur during the pretreatment with formation of the AD inhibiting phenolic degradation products such as syringaldehyde and vanillin (Barakat et al., 2012).

2.5.4 Inhibitory and toxic compounds from pyrolysis

APL contains several toxic compounds and AD containing mixed anaerobic consortia possible of adaptation to a wide range of chemical substances (Appels et al., 2011) can be exploited for its capacity to degrade and convert such to methane (Torri and Fabbri, 2014; Wen et al., 2020).

Organics with molecular weight less than 1000 in HTL aqueous phase has been degraded in AD batch tests (Chen et al., 2016).

Si et al. (2018) observed complete conversion of furfural and HMF to methane while some phenolic compounds such as 4-ethyl-phenol and 3-hydroxypyridine degraded with some inhibition in acetogenesis during continuous AD.

Pyridine and pyridine derivatives can also be converted by AD at low biodegradation rates (Li et al., 2001). Pyridine degradation can be enhanced by nitrate as electron acceptor (Shen et al., 2015a). Sun et al. (2011) observed a synergistic effect of the inhibitors found in APL, in which phenol > 400 mg/L inhibited pyridine degradation. However, inhibition caused by phenol on pyridine degradation was less in immobilized compared to suspended cultures (Kim et al., 2006) implying that microbial aggregates (e.g. biofilm and granules) may handle APL toxins better than reported batch tests.

2.6 Pretreatment tuning to increase overall anaerobic digestion yield

Pretreatment conditions should be chosen based on primary objective while also considering the utilization of bi-product streams for enhanced resource recovery. The whole process should be optimized according to the total set of products, including methane production by AD of the liquid. In this perspective, efficient pretreatment must extract hemicellulose while minimizing carbohydrates loss and limiting inhibitors formation in the total process (Benjamin et al., 1984) so that the hydrothermal extraction can produce value-added products, such as biogas, from compounds that are otherwise wasted in other pretreatment schemes (Liu, 2015).

2.6.1 Hot water extraction

The hydrolysate composition after HWE vary depending upon the origin of lignocellulosic biomass and process conditions such as reaction temperature, solid to liquid ratio, type of reaction vessel and mode of operation, i.e, batch or continuous (Ahmad et al., 2018).

Temperature affects the extraction mass removal (Lu et al., 2012), where an optimal extraction severity can be observed. E.g. HWE of sorghum sundanense treated at 100 °C for 1 hour produced the highest yield (0.282 m^3 CH_4/kg VS) of the conditions tested and treatment at lower and higher temperatures led to lower AD methane yield (Sambusiti et al., 2013).

A higher extraction pressure maintains the liquid hot water as solvent to solubilize mainly the hemicellulose, which makes the cellulose better accessible for microorganisms and reduces the formation of inhibitors (Hendriks and Zeeman, 2009; Liu, 2015). pH can also influence the formation of inhibitors and should be maintained between 4 and 7 (Mosier et al., 2005a). This optimized pH also maximizes the solubilization of the hemicellulose fraction and reduces the formation of monomeric sugars and therefore also the formation of inhibitory degradation products (Mosier et al., 2005a).

2.6.2 Pyrolysis

Pyrolysis parameters should be tuned for higher APL yield if the side stream is to be treated by AD, implying HTL with moderate temperature (around 400 °C) and short vapor residence time, producing bio oil. This will increase easily degradable ketones and acids and decrease the concentration of recalcitrant or toxic carbon compounds such as hydroxyacetaldehyde, acetol, furans, N-heterocyclic compounds and phenols in APL for enhanced methane yield (Alvarez et al., 2014; Hierholtzer et al., 2013; Rezaei et al., 2014). Parameter tuning should be chosen accordingly if biochar or syngas production is also considered, where lower process temperature and longer vapor residence time favors char production and high temperature and longer residence time increases syngas formation (Bridgwater, 2012).

Physical, thermal, chemical and biological pretreatments on biomass before pyrolysis can also help in avoiding inhibitors and increasing sugar concentration in the APL, to favor AD methane production. HWE before pyrolysis removes hemicellulose and alkali metals (Na and K) leading to increased sugar concentration, mainly levoglucosan, while reducing possible AD inhibitors such as acetic acid, carboxylic acids, ketones and phenols in the bio-oil (APL and organic phase) (Chang et al., 2013; Kan et al., 2016; Tarves et al., 2017). Similarly, steam explosion pretreatment also reduces acids, furfural and HMF concentration in the bio-oil (APL and organic phase) while increasing the concentration of levoglucosan and other anhydrous (Hassan el et al., 2009; Kan et al., 2016; Wang et al., 2011) increasing the methane production (Liaw et al., 2020). Comprehensive reviews on details of such pre-treatments are published by Alvarez-Chavez et al. (2019) and Kumar et al. (2020).

3 Materials and methods

A brief overview of the pre-treatments used in the feed preparation, materials and methods is included here. Detailed descriptions are available in the attached articles in part II.

3.1 Feed Preparation

Feeds used in AD are prepared at RISE-PFI, Trondheim, Norway. Hydrolysate from hot water extracted Norway spruce and birch and APL from pyrolysis of Norway spruce and birch (with and without HWE) was sent for AD experiments at University of South-Eastern Norway (USN), Porsgrunn.

3.1.1 Raw materials

Woodchips of Norway spruce and birch were used for this experiment. The samples were air dried to 93-94 % DM and fractionated to a size between 13 mm and 5 mm.

3.1.2 Hot water extraction

The wood chips were hot water extracted in a Mini-Mill Laboratory Digester (MMLD) where a temperature of 140 ℃ or 170 ℃ was kept for 300 min or 90 min, respectively. The temperatures and retention times were chosen based on literatures (Liu, 2015; Nitsos et al., 2016), so as to extract maximum hemicellulose from the biomass without the formation of AD inhibitors in the hydrolysate while also pretreating the solid residues to be used in bio-char production. After the HWE, the hydrolysate was collected, analyzed and tested for AD.

In order to describe the combined effects of pretreatment time and temperature for each pretreatment, a severity factor (Eq. 1) (Overend and Chornet, 1987) is calculated for each hydrolysate (Table 3.1).

$$\text{Severity factor} = \log\left(R_0\right) = \log(t \times \exp\left(\frac{T-100}{14.75}\right)) \tag{1}$$

Where, T (°C) is the pretreatment temperature and t (min) is the reaction time.

Table 3.1 Severity factor for the hydrolysates from HWE

Hydrolysate samples	Hydrothermal pretreatment conditions		Severity factor ($\log(R_0)$)
	Temperature (°C)	Time (min)	
Norway spruce170/birch170	170	90	4.02
Norway spruce140/birch140	140	300	3.65

3.1.3 Synthetic hydrolysate

Corresponding synthetic hydrolysates were prepared to closely simulate real hydrolysate based on sugar concentrations and acetic acid while excluding inhibitors (e.g., furfural, HMF, soluble lignin) content to study their effects on methane yield and methane production rate. Synthetic hydrolysates also acted as positive control.

3.1.4 Pyrolysis

The wood chips (with or without HWE) were pyrolyzed in a pyrolysis development unit at RISE PFI AS (Figure 3.1). The Pyrolysis Development Unit consists of a stainless-steel fix bed reactor of 5.6 L located in an oven. A heated gas transfer line connects the reactor with a condensation unit, consisting of two water-cooling condensers, an electrostatic precipitator (ESP) and a silica gel filter. The vapours condensed in the water condenser naturally phase separated into an organic phase and an aqueous phase. The condensate was stored in the condensate bottles overnight before the top phase was decanted off. The aqueous phases produced were used as feed for AD.

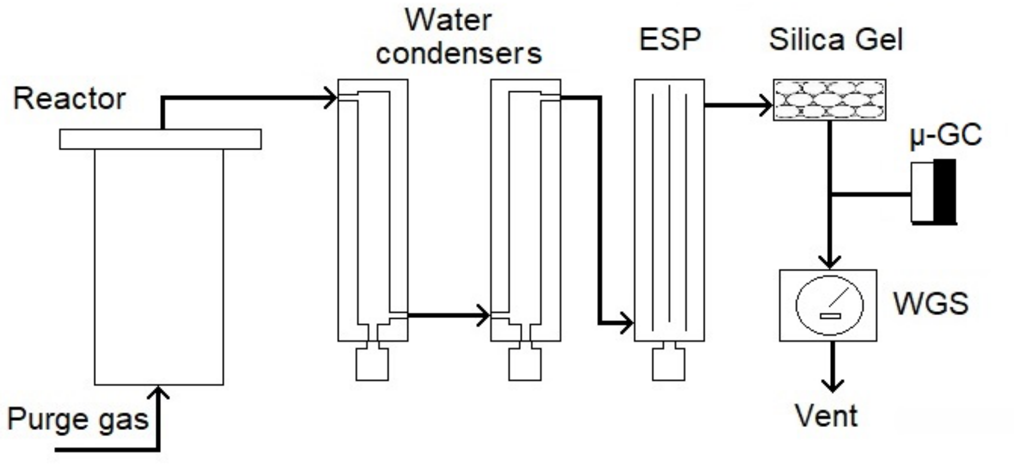

Figure 3.1: Sketch of the pyrolysis rig at RISE PFI.

3.2 Anaerobic digestion

3.2.1 Batch reactors

3.2.1.1 Automatic methane potential test system (AMPTS II)

Automatic Methane Potential Test System II (AMPTS II, Bioprocess Control® Sweden AB) is a standardized laboratory set-up which automatically determines methane potential of any biodegradable material by the water displacement method. It is equipped with a heat bath, automatic stirrer motors and a CO_2 removal unit (Figure 3.2). The system is monitored via software provided by the company.

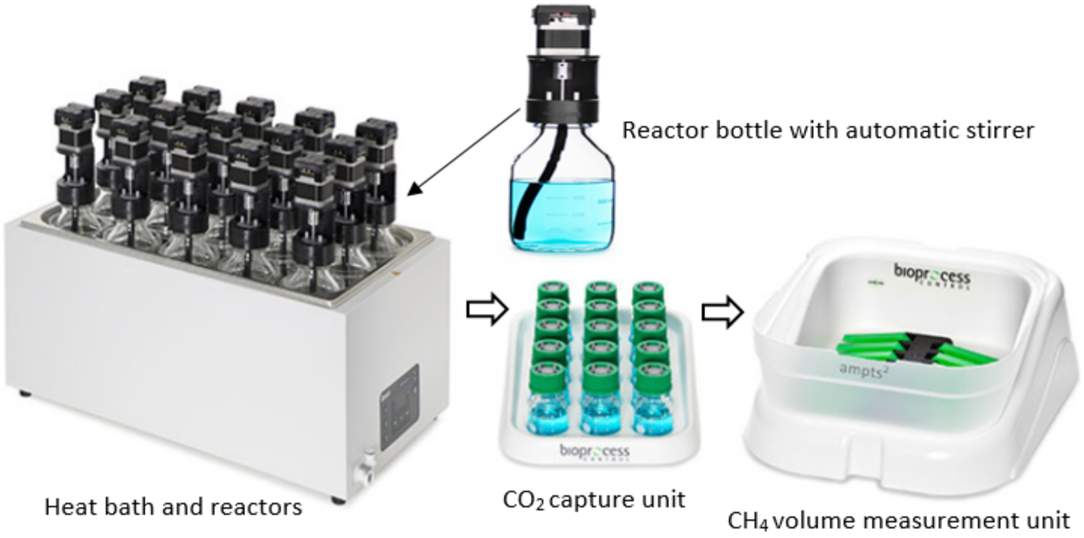

Figure 3.2: AMPTS II set-up and equipment description (figure from Bioprocess control's homepage).

3.2.1.2 Syringe test

Disposable plastic medical syringes (BD Plastipak, Franklin Lakes, NJ, USA) of 100 mL are used as batch reactors to study the effect of OL on methane yield. Anaerobic condition is maintained by removing air and sealed by a needle with silicone rubber stopper at the tip (Figure 3.3). Biogas volume is measured by piston displacement in the syringe and is collected and analysed for methane content.

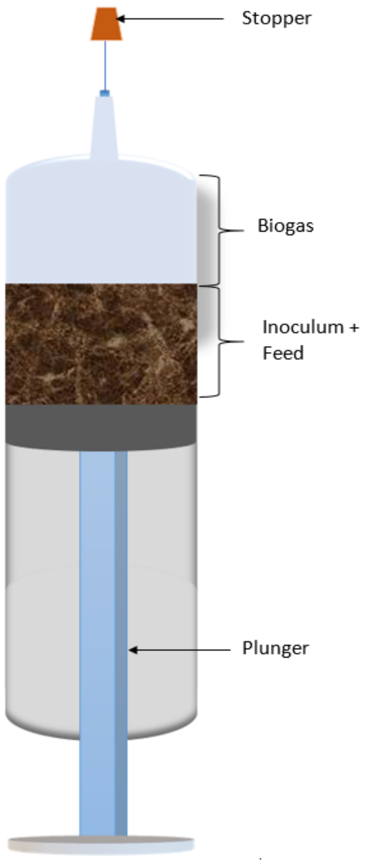

Figure 3.3: Syringe batch reactor test set-up with 100 mL medical syringe.

3.2.2 Continuous fed reactor (Unpublished)

A simplified UASB reactor (Figure 3.4a) of a 370 mL glass vessel with 345 mL working volume (Bergland et al., 2015) was used in the experiment. The reactor was 130 mm high and 60 mm wide with substrate inlet through a central tube ending around 10 mm above the bottom of the reactor. A wide circular plate was installed at the end of the inlet tube to distribute feed uniformly below the sludge bed. The lab-scale process line is shown in Figure 3.4b. Biomass is retained inside the reactor by a solid separator installed inside the reactor while the liquid and gas are separated outside the reactor for efficient operation. The substrate tank was kept in a cooler (4 °C) while four simplified UASB reactors were kept in a water bath to maintain mesophilic condition (35 °C).

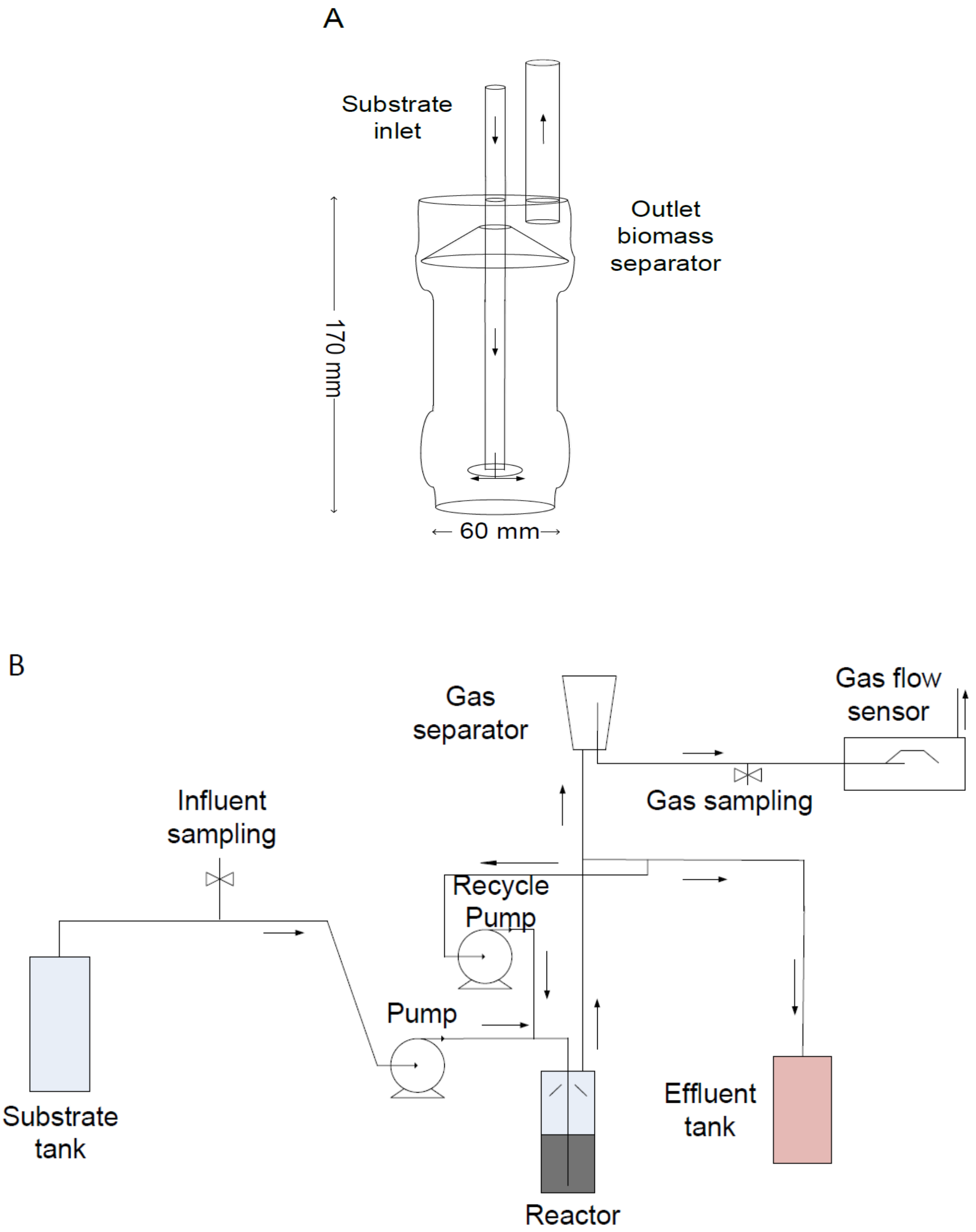

Figure 3.4 A) Sketch of lab-scale AD reactor with influent and effluent pipes and separator. B) Diagram of schematic process line of lab-scale UASB.

About half of the reactor volume was filled with granular sludge (same as used in batch tests). Four identical reactors were operated where two reactors were fed with hydrolysates pretreated at 170 °C while the other two reactors were fed with hydrolysates pretreated at 140 °C. A recirculation flow was maintained at 4 L/d to keep the granular sludge bed suspended. The feed was fed in pulse 24 times per day, feeding rate increasing over time with increasing OLR. Initially 45 mL/d of feed was pumped into the reactors giving OLR of 3.23 kg COD/(m^3 d) and hydraulic retention time (HRT) of 7.7 days for hydrolysate pretreated at 140 °C and OLR of 4.10 kg COD/(m^3 d) and HRT of 7.7 days for hydrolysate pretreated at 170 °C. OLR was increased gradually and values of pH, COD removal and biogas production were observed.

Initially the feed pH was maintained at 6.5 using 2M NaOH but after day 63, feed with pH as low as 5.6 was pumped into the reactors.

The feed was added macronutrients and micronutrients. A macronutrient solution was made of NH_4Cl (44.48 g/L), $(NH_4)H_2PO_4$ (5.3 g/L), $(NH_4)_2HPO_4$ (1.78 g/L), $MgCl_2{\cdot}6H_2O$ (21.4 g/L), $CaCl_2{\cdot}2H_2O$ (7.56 g/L) and $NaHCO_3$ (100 g/L). Similarly, the micronutrients were prepared from Yeast Extract (2.5 g/L), $FeCl_3{\cdot}6H_2O$ (0.2 g/L), $ZnCl_2$ (5.2 g/L), $MnCl_2{\cdot}4H_2O$ (0.0472 g/L), $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (0.064 g/L), $AlKO_8S_2{\cdot}12H_2O$ (0.01 g/L), $CoCl_2{\cdot}6H_2O$ (0.2 g/L), $NiCl_2{\cdot}6H_2O$ (0.52 g/L), H_3BO_3 (0.12 g/L), $CuCl_2{\cdot}2H_2O$ (0.32 g/L) and HCl (20 mL/L). The macronutrient solution was added to maintain a minimum COD:N:P ratio of 350:5:1 (Baeta et al., 2013), while 4 mL micronutrient solution was added per 1000 L feed.

3.2.3 Integration of hot water extraction and anaerobic digestion

In this integration (Article 1 and 2), hydrolysates of Norway spruce and birch pretreated at 140 °C and 170 °C are anaerobically digested to evaluate the effects of pretreatment conditions and biomass types on methane yield from hydrolysates. Two AD temperatures are utilized.

3.2.4 Integration of pyrolysis and anaerobic digestion

In this integration (Article 3), APL produced by pyrolysis of Norway spruce and birch (without HWE) at two temperatures (400 °C and 550 °C) is digested anaerobically to evaluate the effects of pyrolysis temperature and biomass types on methane yield from APL.

3.2.5 Integration of hot water extraction, pyrolysis and anaerobic digestion

3.2.5.1 Anaerobic digestion of aqueous pyrolysis liquid from hot water extracted lignocellulosic biomass

In this integration (Article 4), Norway spruce and birch are hot water extracted at 140 °C and 170 °C before pyrolysis at 400 °C (Figure 3.5). APL, thus obtained, is anaerobically digested to evaluate the effect of HWE temperatures on methane yield from APL compared to APL without HWE.

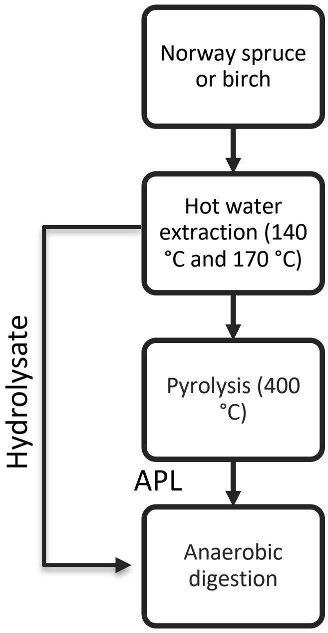

Figure 3.5: Integration of APL from pyrolysis of hot water extracted lignocellulosic biomass and AD.

3.2.5.2 Anaerobic co-digestion of aqueous pyrolysis liquid from hot water extracted lignocellulosic biomass and hot water extract (Cascade process)

In this integration (Article 4), APL and hydrolysate produced in section 3.2.5.1 are co-digested in two different COD based ratios of 3:1 and 1:1 (Hydrolysate:APL) to study the effect of co-digestion on methane yield compared to individually digested hydrolysate and APL.

3.3 Analytical methods

3.3.1 Hot water extraction

The organic content of samples was determined as soluble (filtered through 0.45 µm pore size glass filter) and total COD values according to American public health association (APHA) standard method 5220D using commercial kits (WTW™) and Spectrophotometric method (APHA, 1995) in Spectroquant Pharo 300 spectrophotometer (Dramstadt, Germany). The carbohydrate composition was analyzed according to the NREL procedure by Sluiter et al. (Sluiter et al., 2010), using a Dionex ICS500 High-performance liquid chromatography (HPLC) system from ThermoFisher Scientific. Approximations of the furfural and HMF concentrations in the hydrolysates was done according to the procedure of Chi et al. (Chi et al., 2009), using a UV 1800 from Shimadzu.

3.3.2 Pyrolysis

Water content was measured in all APL with Karl-Fischer titration on a Mettler Toledo V20 Volumetric KF Titrator. Carbon content of the APL was analysed on a Thermo Scientific Flash 2000 CHNS/O Analyzer.

3.3.3 Anaerobic digestion

VFA concentrations were carried out using gas chromatography HP 6890 serial C (Hewlett-Packard) with a flame ionization detector and a capillary column (DB-FFAP 30 m long and 0.25 mm ID, 0.25 µm film). Helium was used as the carrier gas at a flow velocity of 5 mL/min with detector gases as hydrogen and air. The injector and the detector temperatures were set to be 200 °C and 250 °C, respectively. The oven was programmed to start at 80 °C and hold for 1 min, then to 180 °C at a rate of 30 °C/min, then to 230 °C at a rate of 100 °C/min. Biogas compositions of samples were analyzed using SRI 8610C Gas chromatography using Helium as a carrier gas and oven temperature kept constant at 83 °C, calibrated with a standard mixture of 60 % methane and 38 % carbon dioxide. pH was measured using Beckman 300 pH meter equipped with Sentix-82 pH electrode or WTW™ inolab pH7110.

4 Results and discussions

An overview of results obtained during the AD of hydrolysate and APL leading to scientific papers (listed in part II) as well as unpublished results are presented and discussed in this chapter.

4.1 Anaerobic digestion of hydrolysate (Article 1, 2, 4 and unpublished results)

The effect of pretreatment conditions (temperature and retention time) and types of biomass (hardwood (birch) and softwood (Norway spruce)) on batch methane production from hydrolysate is evaluated in sub-section 4.1.1. Similarly, the effects of OL and AD temperature on methane production in batch reactors has been summarized in sub-section 4.1.2 and 4.1.3, respectively, and mesophilic continuous AD (unpublished) is summarized in sub-section 4.1.4 taking Norway spruce hydrolysate as feed. The work is presented in articles 1, 2 and 4.

4.1.1 Effect of pretreatment severity and biomass types on methane yield and rate from Norway spruce and birch hydrolysate

Results:

During AMPTS II batch tests, methane yields from hydrolysates pretreated at 140 °C were higher compared to the hydrolysates pretreated at 170 °C, during both mesophilic and thermophilic conditions at the organic loading of 10 and 20 g COD/L (Figure 4.1). Birch (hardwood) hydrolysate pretreated at 140 °C had 15 % more methane yield compared to hydrolysate pretreated at 170 °C while for Norway spruce (softwood) the increase was not significant (3 %) during mesophilic AD at OL of 10 g COD/L. While comparing Norway spruce hydrolysate results, the methane yield values during mesophilic conditions at OL of 20 g COD/L were significantly lower than the methane yields at OL of 10 g COD/L. Methane yield of Norway spruce hydrolysate pretreated at 170 °C during the OL of 20 gCOD/L was 42 % lower compared to only 18 % of hydrolysate pretreated at 140 °C, suggesting that the effect of pretreatment temperature was more pronounced at higher OLs.

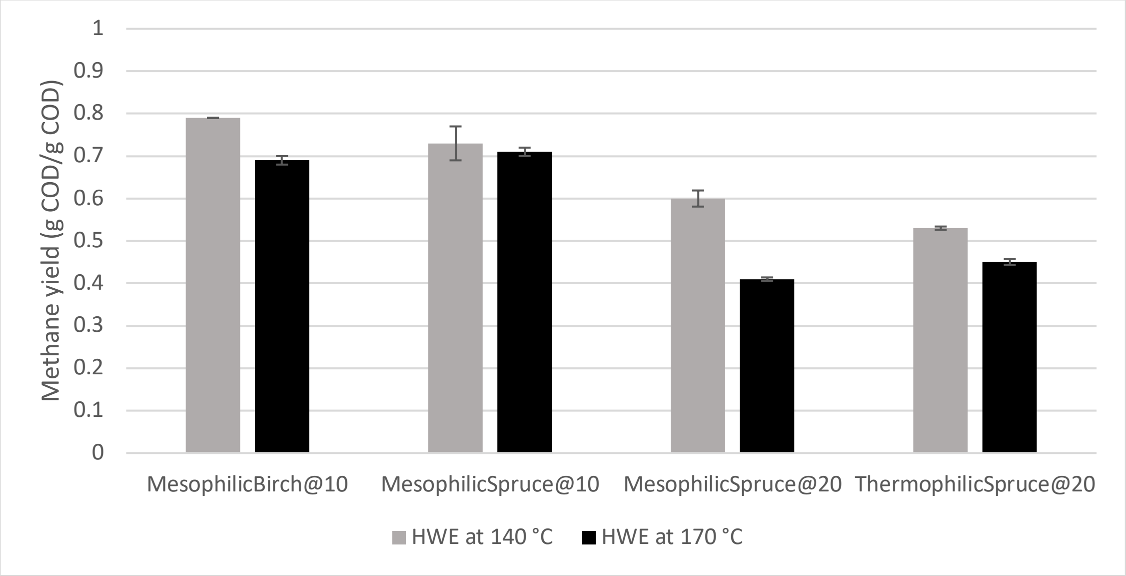

Figure 4.1 Methane yields of Norway spruce and birch hydrolysates, pretreated at 140 °C and 170 °C, under mesophilic and thermophilic conditions at organic load of 10 and 20 g COD/L in AMPTS II.

Discussion:

The lower methane yield of hydrolysate pretreated at 170 °C compared to 140 °C in most of the cases during AD batch tests can be attributed to higher concentration of inhibitors as higher pretreatment temperature that leads to higher sugar dissolution which increases the concentration of AD inhibiting sugar degradation products such as furfural and HMF (Monlau et al., 2014). Hydrolysate pretreated at 170 °C is also expected to have higher concentration of complex recalcitrant compounds and soluble lignin than pretreatment temperature at 140 °C which remain undigested, is slowly degraded or acts as inhibitor in different steps of AD (Benner et al., 1984; Jimenez et al., 1990; Koyama et al., 2017). Higher concentration of inhibitors and soluble lignin also led to longer lag phase between the two methane production peaks compared to the pretreatment temperature of 140 °C indicating longer time taken by the microorganism to adapt to the oligomers and complex compounds broken down during the second peak (Benjamin et al., 1984) and also possibly slow breakdown of some part of soluble lignin (Benner and Hodson, 1985).

Higher methane yield in birch (hardwood) hydrolysate pretreated at 140 °C compared to Norway spruce (softwood) can be attributed to better hemicellulose extraction of hardwood due to its higher content of hemicellulose, type of hemicellulose (acetylated xylans), lower lignin content and the more complex nature of the lignin component to extract (Pan et al., 2005; Vivekanand et al., 2013). However, higher hemicellulose concentration in hardwood also leads to higher degradation product concentration at high pretreatment temperature with products hampering the methane yield as observed in birch pretreated at 170 °C. Higher concentration of furfural (0.66 g/L) in birch hydrolysate pretreated at 170 °C gave slightly lower methane yield as observed by others at nearly similar concentration (Ghasimi et al., 2016). Synergistic inhibition effect from individual inhibitors may also have affected the methane yield (Mussatto and Roberto, 2004).

4.1.2 Effect of organic load during anaerobic digestion of Norway spruce hydrolysate

Results:

During mesophilic syringe batch tests, increasing OL beyond 10 g COD/L had significant effect on the methane yield of hydrolysate pretreated at 170 °C, unlike hydrolysate pretreated at 140 °C (Figure 4.2). Hydrolysate pretreated at 140 °C had no effect of OL on methane yield and the values were around 0.7 g COD/g COD for all OLs. During thermophilic syringe batch tests, increasing OL of the both hydrolysates pretreated at 170 °C and 140 °C had similar trend of negative effect on the methane yield.

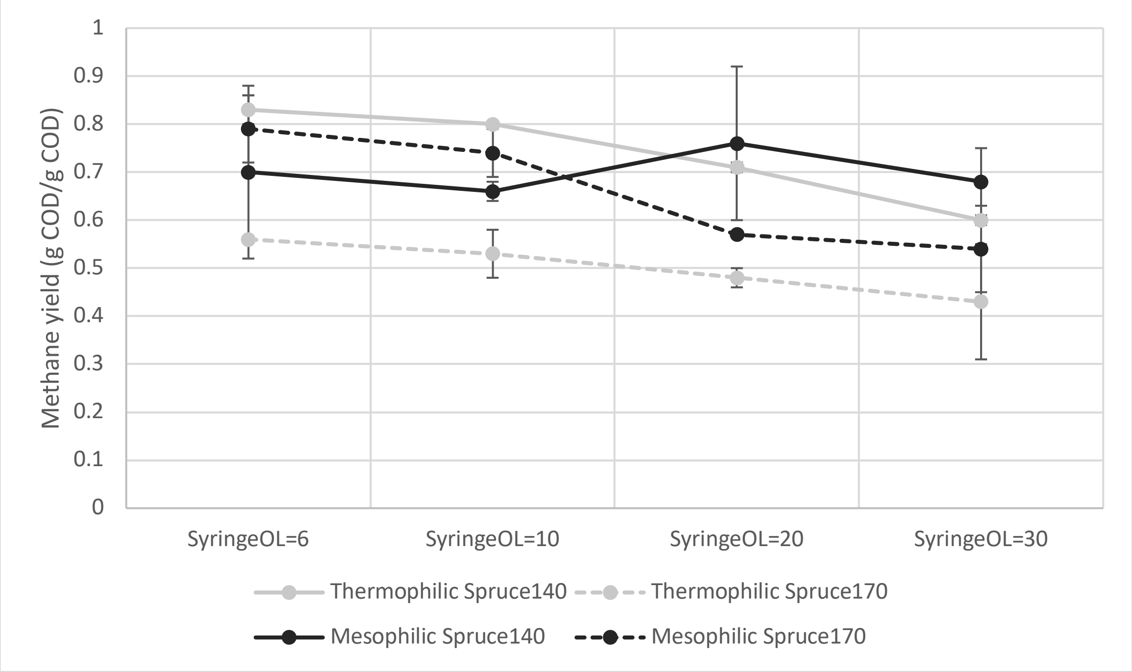

Figure 4.2 Variations of methane yield of Norway spruce hydrolysates, pretreated at 140 °C and 170 °C, during mesophilic and thermophilic conditions at organic loadings of 6, 10, 20 and 30 g COD/L.

Discussion:

Hydrolysate pretreated at 140 °C seems to be better suited for higher OLs compared to hydrolysate pretreated at 170 °C in both thermophilic and mesophilic conditions. A decrease in methane yield with increase in OL can be explained by inhibition from the increased stress on microorganisms due to imbalance between VFA production and its consumption and the increasing effect of inhibitor at increased OLs. Since high OL during mesophilic condition did not compromise methane yields from hydrolysate pretreated at 140 °C, lower methane yield (32 %) of hydrolysate pretreated at 170 °C can be attributed to inhibition only from inhibitors such as furans and possible soluble lignin and lower methane yield (19 %) of hydrolysate pretreated at 140 °C compared to the synthetic hydrolysate (positive control) is primarily due to recalcitrant compounds and not inhibition.

4.1.3 Effect of anaerobic digestion temperature of Norway spruce hydrolysate

Results:

During thermophilic condition the methane yield from syringes (Figure 4.2) of hydrolysate pretreated at 140 °C was hampered by increased OL unlike mesophilic condition. Thermophilic condition also resulted in a significantly lower methane yield of hydrolysate pretreated at 170 °C compared to mesophilic condition.

During AMPT II tests thermophilic AD (55 °C) of Norway spruce hydrolysate pretreated at 140 °C resulted in lower methane yield (12 %) than mesophilic AD (35 °C) during the OL of 20 g COD/L. However, thermophilic AD had higher methane yield (10 %) than mesophilic AD when the hydrolysate was pretreated at 170 °C (Figure 4.3).

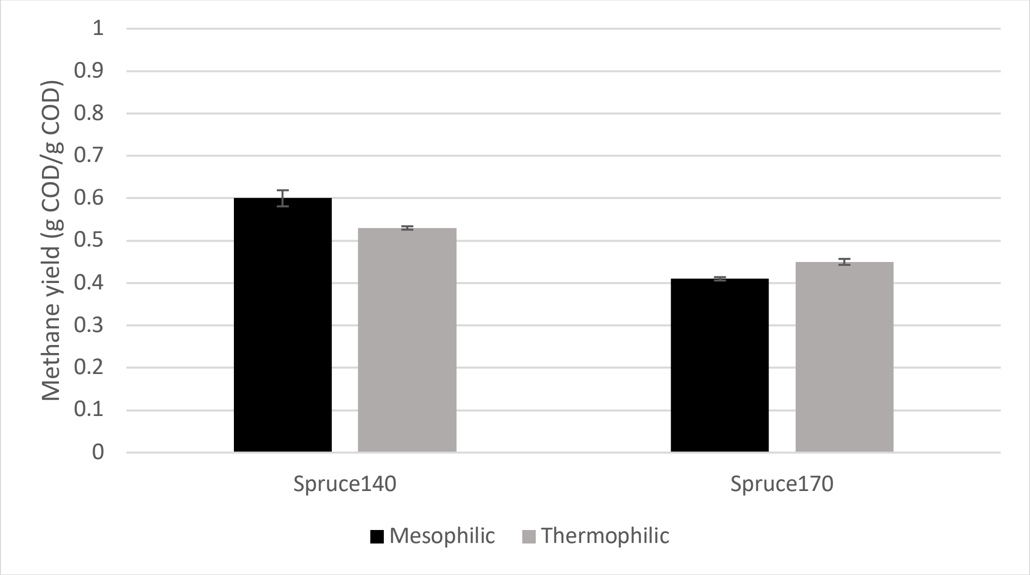

Figure 4.3 Methane yield of hydrolysates of Norway spruce pretreated at 140 °C and 170 °C under mesophilic and thermophilic AD conditions.

Methane production rates of Norway spruce hydrolysates were higher during mesophilic condition compared to thermophilic condition, and hydrolysates pretreated at 140 °C had higher methane production rates compared to hydrolysates pretreated at 170 °C during the both mesophilic and thermophilic condition (Figure 4.4).

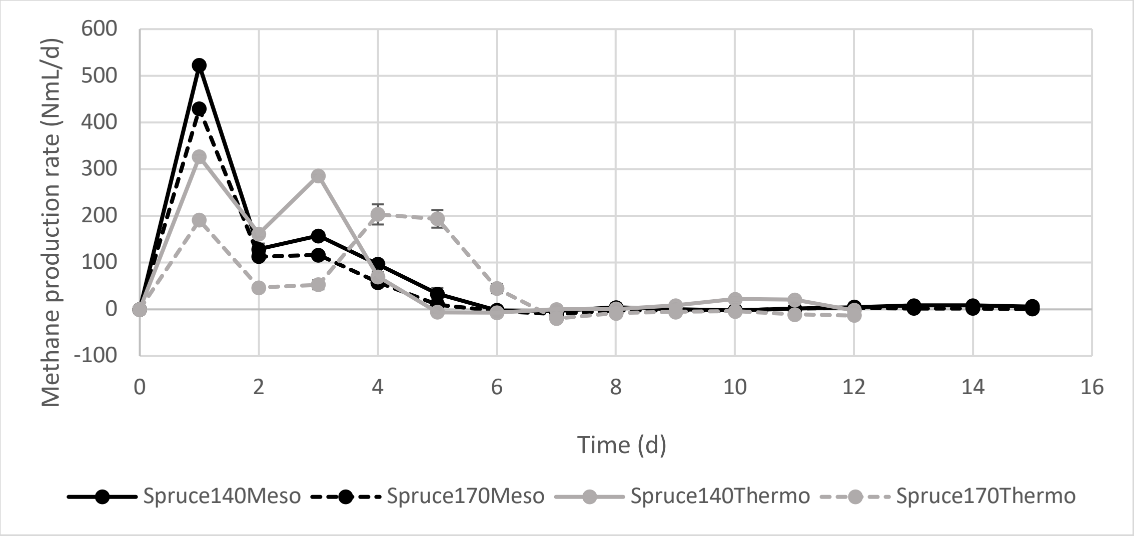

Figure 4.4 Methane production rates of hydrolysates of Norway spruce pretreated at 140 °C and 170 °C under mesophilic and thermophilic AD conditions.

Discussion:

The methane yield of hydrolysate pretreated at 140 °C was hampered by increased OL during syringe batch tests at thermophilic condition unlike mesophilic condition and can be explained by pronounced effect of furan inhibitors during thermophilic condition compared to mesophilic condition (Ghasimi et al., 2016). The lower methane yield is also observed during AMPTS II tests at high load. It was also a significantly lower methane yield during syringe tests of hydrolysate pretreated at 170 °C compared to hydrolysate pretreated at 140 °C at thermophilic condition explained by the pronounced effect of furan inhibitors. The possible effect of furan inhibitors can also be observed during methane production rate as the hydrolysate pretreated at both pretreatment temperatures of 140 °C and 170 °C during thermophilic condition was lower compared to mesophilic condition.

Interestingly, hydrolysate pretreated at 170 °C during thermophilic AD in AMPTS II at OL of 20 g COD/L gave slightly higher methane yield compared to mesophilic condition which was also observed in syringe tests at low OLs for hydrolysate pretreated at 140 °C. This could be explained by improved degradation of soluble lignin at thermophilic condition (Benner and Hodson, 1985) as there are reported several degradation effects of AD temperature (Ghasimi et al., 2016; Benner and Hodson, 1985). However, 53 days adaptation period of mesophilic granular sludge to thermophilic condition in the laboratory might not have been enough resulting in slower rate effects during thermophilic AD process.

4.1.4 Continuous AD of Norway spruce hydrolysates in a high rate reactor (Unpublished results)

Results:

During the AD of Norway spruce hydrolysate pretreated at 140 °C, the start-up was quick and biogas production started immediately. Biogas flow and yield were stable during the start of experiment (from day 0 to 20) when OLR was stable (Figure 4.5). When OLR was increased starting on day 26, biogas production increased accordingly but the yield decreased suggesting overloading conditions. When OLR was reduced to less than 1 kg COD/(m^3 d), the biogas yield started increasing and remained constant at around 0.4 m^3/kg COD with stable reactor performance. The biogas yield remained almost constant when OLR was increased up to 5 kg COD/(m^3 d). However, the biogas yield started reducing below 0.4 m^3/kg COD when OLR was increased above 5 kg COD/(m^3 d) and reduced to 0.22 m^3/kg COD at the highest operative OLR (7 kg COD/(m^3 d)). During the stable operation (day 68 to 130), biogas yield was 0.43±0.06 m^3/kg COD corresponding to methane yield of 0.24 m^3/kg COD or 213 NmL/g COD (taking average value of methane content from 27 samples, 56.1±4.1 %).

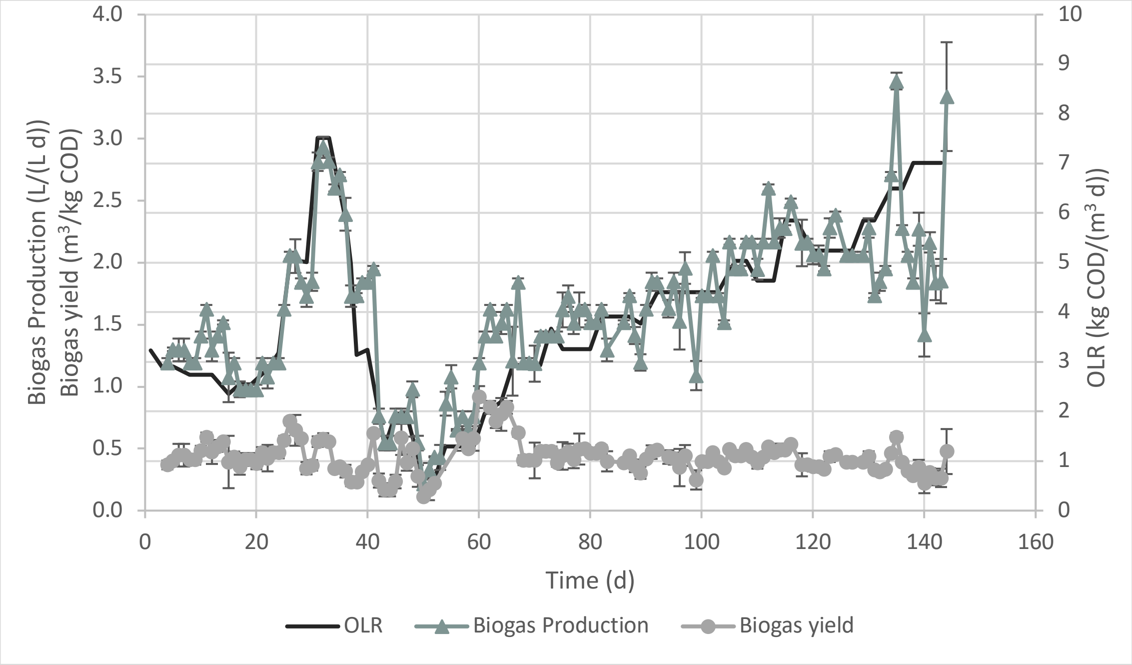

Figure 4.5 Biogas production during OLR increase over time during AD of Norway spruce hydrolysate pretreated at 140 °C.

Similarly, during AD of Norway spruce hydrolysate pretreated at 170 °C, the biogas yield was stable after a slow start (Figure 4.6). During day 8 to 20, the biogas yield was around 0.45 m^3/kg COD but decreased with the increase of OLR to 5.2 kg COD/(m^3 d). Biogas flow increased with the increase of OLR ultimately failing at OLR of 8.7 kg COD/(m^3 d). The reactors failed to recover and had to be restarted again after replacing with new batch of granular sludge. The reactor produced biogas immediately after new start-up at lower OLR. The biogas yield was stable from day 45 to 72 in which biogas yield was 0.39±0.07 m^3/kg COD corresponding to methane yield of 0.21 m^3/kg COD (taking average value of methane content from 15 samples, 54.4±3.44 %). When OLR was increased to 4.5 kg COD/(m^3 d), biogas yield decreased to 0.28 m^3/kg COD and completely failed upon further OLR increase suggesting overloading condition.

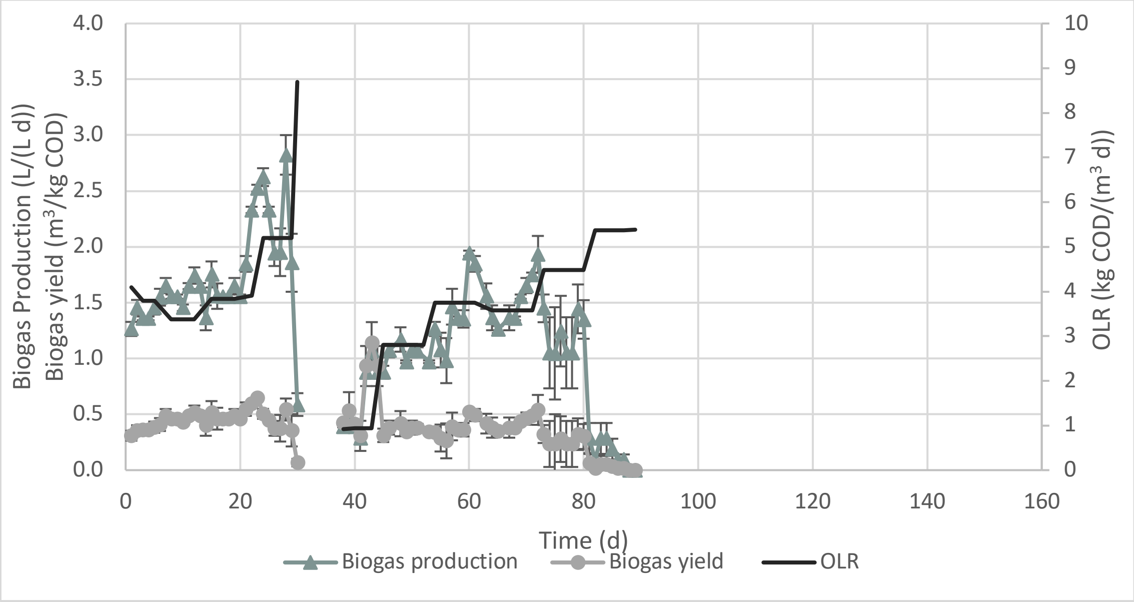

Figure 4.6 Biogas production during OLR increase over time during AD of Norway spruce hydrolysate pretreated at 170 °C.

Discussion:

During the stable operation (day 68 to 130) of hydrolysate pretreated at 140 °C, the methane yield was 213 NmL/g COD which is similar to the batch mesophilic AD value obtained at OL of 20 g COD/L (210 NmL/g COD) but 16 % lower than at OL of 10 g COD/L (254 NmL/g COD). For hydrolysate pretreated at 170 °C during the stable operation (day 45 to 72) the methane yield was 175 NmL/g COD which is 18 % higher than obtained during the batch mesophilic AD at OL of 20 g COD/L (148 NmL/g COD).

Continuous AD of Norway spruce hydrolysates pretreated at 140 °C and 170 °C also revealed the effect of pretreatment temperature on methane yield similar to the results of batch AD. Hydrolysate pretreated at 170 °C had lower methane yield compared to hydrolysate pretreated at 140 °C as in batch AD experiments. Methane yield of hydrolysate pretreated at 140 °C was similar in continuous and batch AD while hydrolysate pretreated at 170 °C had improved methane yield (18 %) during continuous AD and can be attributed to lower OLR compared to batch AD (20 gCOD/L), which had lower inhibition from furan inhibitors and soluble lignin. AD microorganisms might also have adapted to hydrolysate during continuous AD. Failure of the reactor at lower OLR while running hydrolysate pretreated at 170 °C than hydrolysate pretreated at 140 °C also suggests higher inhibition to AD microorganisms. However, replacement of granular sludge leading to lower acclimatization period in reactors running with hydrolysate pretreated at 170 °C could also have played a major role in failure of the reactors at lower OLR compared to reactors running with hydrolysate pretreated at 140 °C.

4.2 Anaerobic digestion of aqueous pyrolysis liquid (Article 3)

Results obtained during the AD of APL prepared from two different types of biomass (birch and Norway spruce) at the pyrolysis temperatures 400 °C and 550 °C are presented, and the effect of pyrolysis temperature and biomass type on methane yield from aqueous pyrolysis liquid is evaluated.

Results:

APL from the pyrolysis used as feedstock in AD gave methane yields depending on the type of biomass and the pyrolysis temperature (Figure 4.7). APL of birch from pyrolysis at 400 °C and 550 °C had a methane yield of 44 % and 49 %, respectively, of theoretical achievable. A small increase in yield was observed with the increase in pyrolysis temperature for birch. Contrarily, a large decrease in methane yield from 59 % to 32 % was observed from the APL of Norway spruce with the increase in pyrolysis temperature from 400 °C to 550 °C. The common trend for both birch and Norway spruce is a decrease in methane yield with increased carbon content in the APL (Figure 4.8).

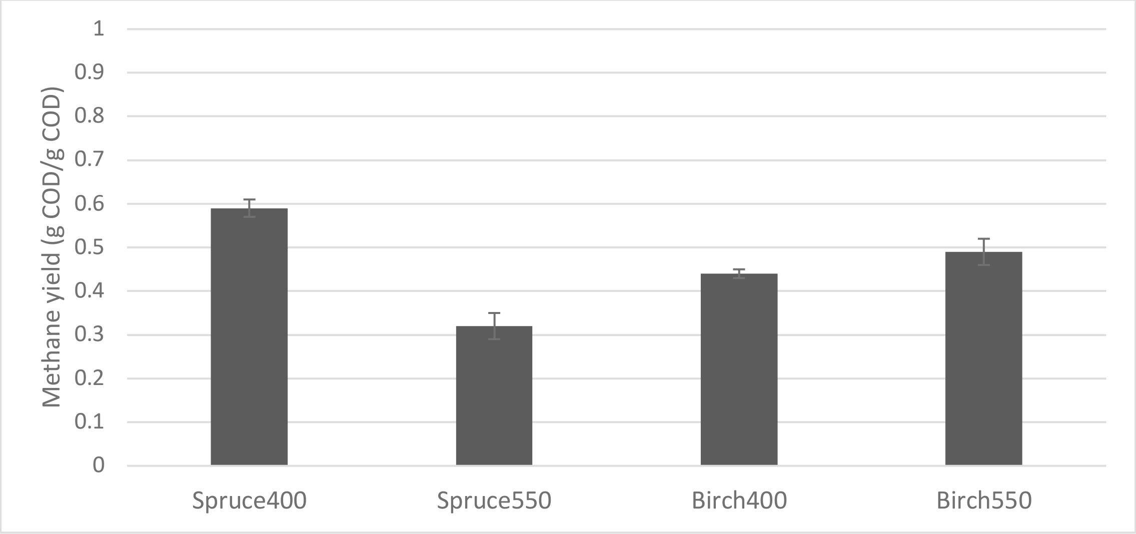

Figure 4.7 Methane yield of APL from pyrolysis of Norway spruce and birch at 400 °C and 550 °C.

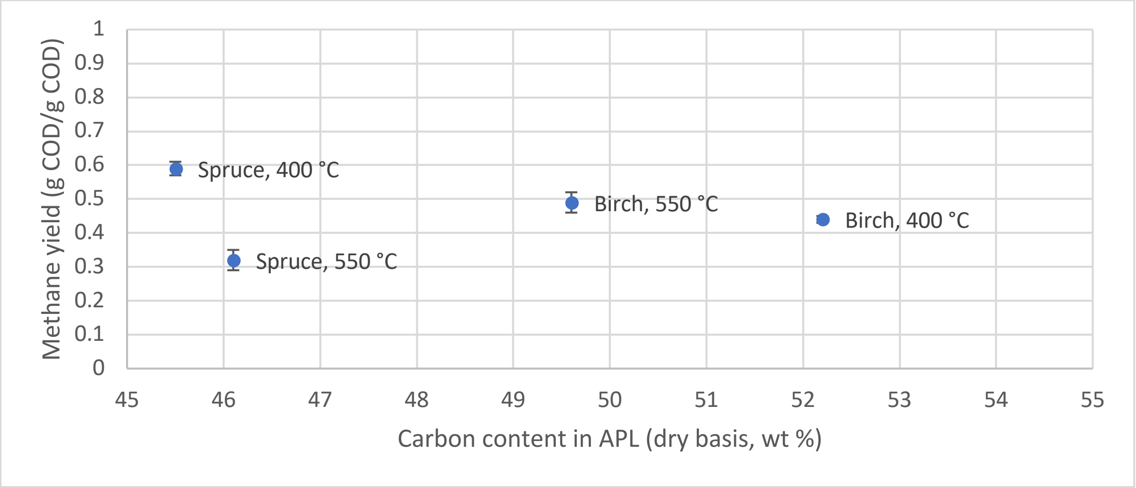

Figure 4.8 Methane yield decreasing with increasing carbon content in APL.

Discussion:

The methane yield of APL from birch and Norway spruce ranged from 32 % to 59 % which is comparable to 52 % of APL of pine wood during continuous AD (Torri et al., 2020). Birch (hardwood) had higher concentration of carbon content in APL (dry basis, wt %) and unlike Norway spruce, the carbon content was higher in APL from pyrolysis temperature of 400 °C compared to 500 °C. The effect of carbon content on methane yield from hardwood APL was lower compared to softwood APL although the difference in carbon content between APL pretreated at different temperatures was significant.

The lower effect of carbon content in methane production from hardwood APL can be explained by the lower lignin content (21 wt. % dry basis) (Părpăriţă et al., 2014) and complex lignin structures (syringil-guaiacyl lignin) of hardwood compared to softwood (guaiacyl lignin) (Fahmi et al., 2008), therefore breaking down at a lower rate yielding less inhibitory APL (Torri et al., 2016). Norway spruce (softwood) had a significant effect of carbon content on methane yield since the lignin content of Norway spruce is high (27.6-29.4 wt. % dry basis) (Părpăriţă et al., 2014). This leads to Norway spruce APL composed of higher concentration of complex phenols with higher molecular weight (Stefanidis et al., 2014).

4.3 Anaerobic digestion of aqueous pyrolysis liquid from pyrolysis of hot water extracted biomass (Article 3 and 4)

Results obtained during the AD of APL prepared from two different types of biomass (birch and Norway spruce) at the pyrolysis temperatures 400 °C after hot water extraction at 140 °C and 170 °C are presented, and the effect of hot water extraction on methane yield from aqueous pyrolysis liquid is evaluated.

Results:

4.3.1 Norway spruce

Methane yield from APL of Norway spruce (softwood) decreased significantly when the biomass was hot water extracted at 140 °C before pyrolysis but the difference was insignificant at HWE of 170 °C (Figure 4.9). Methane yield of 0.59 gCOD/gCOD was observed during the AD of APL of Norway spruce without HWE but methane yield decreased to 0.49 gCOD/gCOD at HWE temperature of 140 °C. The difference in methane yield between pretreatment temperatures of 140 °C and 170 °C was however not significant.

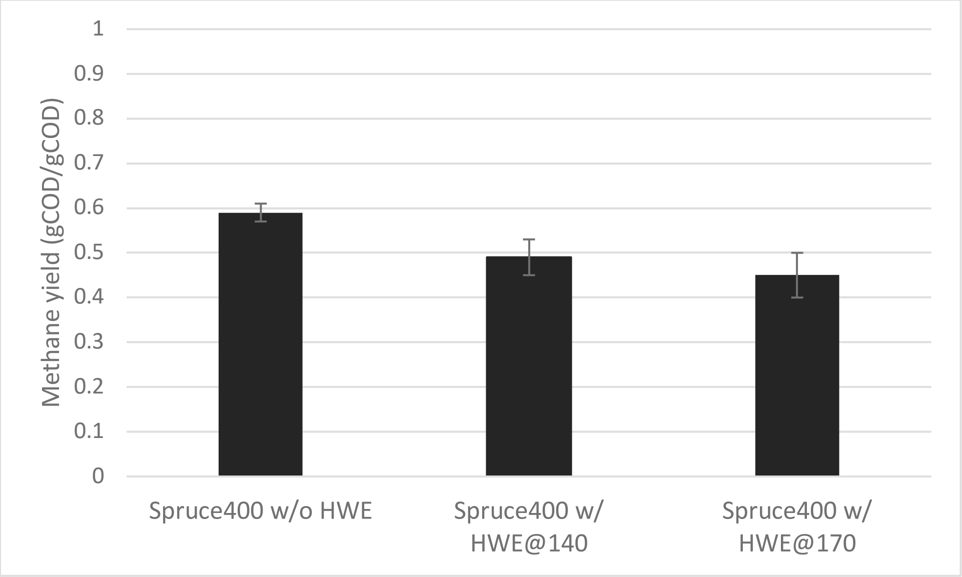

Figure 4.9 Methane yield of APL from pyrolysis of Norway spruce at 400 °C with (at 140 °C and 170 °C) and without HWE.

4.3.2 Birch

HWE before pyrolysis had a significant positive effect on the methane yield from APL of birch (Figure 4.10). Methane yield from APL of birch without HWE before pyrolysis was 0.44 g COD/g COD and the value improved with the increase in HWE temperature to 0.49 g COD/g COD and 0.52 g COD/g COD for 140 °C and 170 °C, respectively. However, the effect of HWE temperatures on the methane yield was not significant.

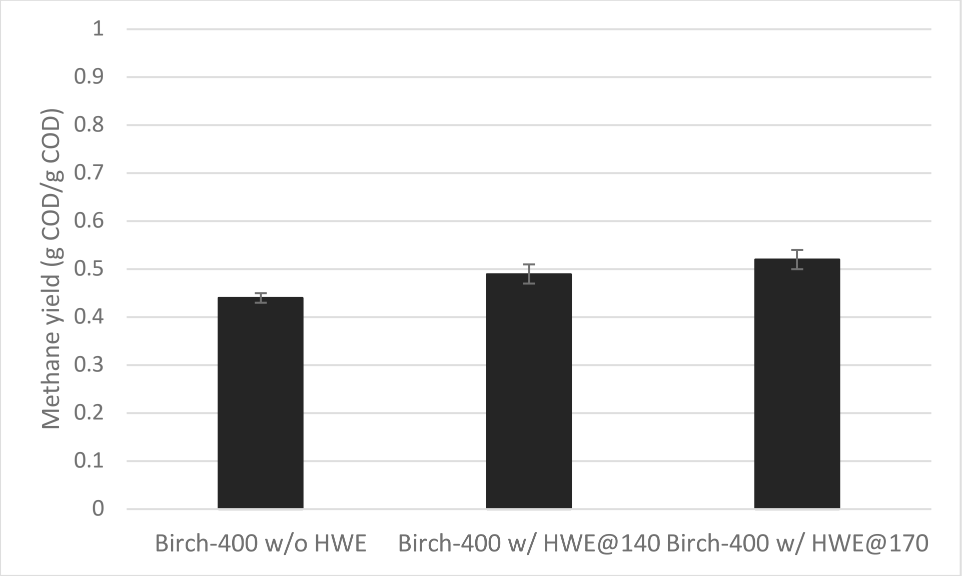

Figure 4.10 Methane yield of APL from pyrolysis of birch at 400 °C with (at 140 °C and 170 °C) and without HWE.

Discussion:

The methane yield of APL from hot water extracted birch improved compared to untreated biomass and can be attributed to the removal of hemicellulose and alkali metals (Na and K) by HWE leading to increased sugar concentration, mainly levoglucosan, while reducing possible AD inhibitors such as acetic acid, carboxylic acids, ketones and phenols in the bio-oil (Chang et al., 2013; Kan et al., 2016; Tarves et al., 2017). On the other hand, the methane yield of APL from hot water extracted Norway spruce at 140 °C decreased compared to APL from untreated Norway spruce. The inconsistency might be due to different biomass types and is beyond the scope of this research. Although the tested HWE temperature before pyrolysis had no significant effect on the methane yield, the digestion period was longer in the case of APL from hot water extracted biomass at low temperature as lower amount of hemicellulose and lignin were extracted from the biomass during low HWE temperature which might have increased the concentration of furan and phenolic compounds in the APL during pyrolysis.

4.4 Anaerobic co-digestion of hydrolysate and APL (Article 4)

Co-digestion of the two waste streams of hydrolysates and corresponding APL, creating a cascade processing, is shortly presented and evaluated in this section.

Results:

A co-digestion ratio of 3:1 (Hydrolysate:APL) improved the methane yield by 40 % and 6 % in Norway spruce and 26 % and 59 % in birch pretreated at 140 °C and 170 °C respectively compared to the 1:1 ratio cases. At the 3:1 ratio, the yield was higher than the sum yield of individual substrates, except Norway spruce pretreated at 170 °C. Similarly, regression analysis showed that the relationship between methane yield and volume of APL was relatively strong (R^2=0.73) (Figure 4.11). During the co-digestion ratio of 1:1, the methane yield in all the samples was found to be significantly lower than the sum yield from the individual substrates (APL and hydrolysate). However, at the 3:1 ratio the yield was not significantly different than the sum yield of individual substrates.

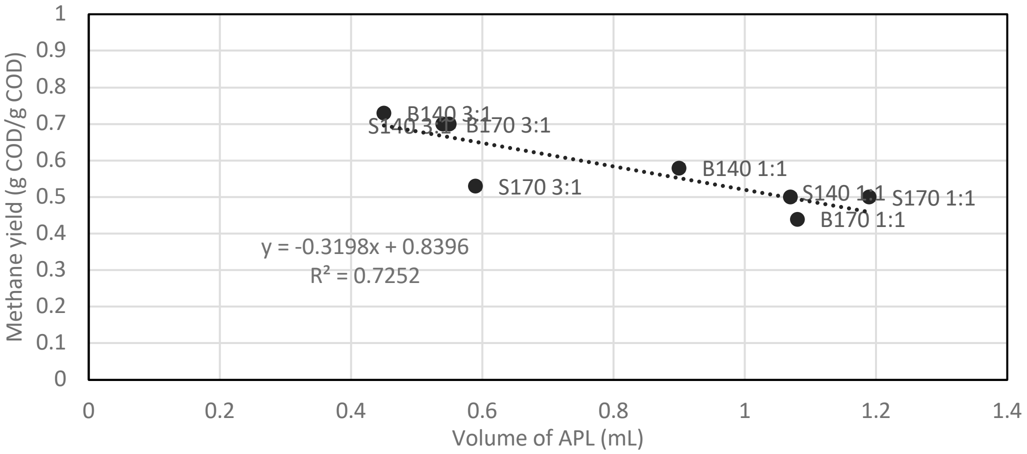

Figure 4.11 Variation of methane yield with amount of APL during co-digestion with hydrolysate.

Discussion:

Diluting APL with a higher amount of sugar rich hydrolysate improved methane yield by reducing toxicity and inhibition of APL which was also observed in reduced digestion period, an absence of lag phase during co-digestion ratio of 3:1 compared to 1:1. APL has also been previously co-digested with swine manure as an additive to observe improved methanogenic activity (Yu et al., 2020). Relatively strong relationship during regression analysis implies that the amount of APL should be carefully controlled for enhanced methane yield during the co-digestion.

5 Conclusions

Innovative cascade processing of hot water extraction before pyrolysis followed by co-digestion of the corresponding waste streams hydrolysate and APL during AD is presented, evaluated and found to be a promising approach. The cascade processing was found to be an effective method for methane production in batch reactors while the hydrolysate was also run in continuous high rate AD reactor and found to handle a wide range of OLRs supported by values of pH, COD removal and biogas production.

The overall research finding of effects of biomass types, HWE pretreatment severity, pyrolysis temperature, AD temperature and OL, HWE before pyrolysis and co-digestion (hydrolysate and APL) on methane production is summarized in this chapter. Further recommendations to understand underlying mechanisms of AD of hydrolysate and APL are also summarized at the end of the chapter.

5.1 Effect of HWE pretreatment severity and biomass types on anaerobic digestion

- AD was found to be an effective method for methane production from organic rich waste streams of birch and Norway spruce hydrolysate as feedstock with biodegradability ranging from 69 to 79 % in batch reactors and 50 to 61 % in continuous reactors.
- Birch hydrolysate pretreated at 140 °C had 15 % more methane yield than birch hydrolysate pretreated at 170 °C and is attributed to lower concentration of inhibitors and possibly soluble lignin. For Norway spruce hydrolysate the difference in methane yield was insignificant between pretreatment at 140 °C and 170 °C in batch AD, but hydrolysate pretreated at 140 °C gave higher methane yield during continuous AD.
- Pretreatment at 140 °C resulted in higher methane yield from birch hydrolysate than Norway spruce, with opposite result from pretreatment at 170 °C which gave lower methane yield from birch hydrolysate compared to Norway spruce suggesting higher pretreatment temperature leads to increased sugar

degradation products in birch compared to Norway spruce, inhibiting the AD process.

5.2 Effect of organic load and temperature during anaerobic digestion of Norway spruce hydrolysate

- During mesophilic AD, increasing OL beyond 10 g COD/L had a significant negative effect on the methane yield of Norway spruce hydrolysate pretreated at 170 °C, unlike 140 °C that had slightly lower methane production rate at high OL but without a compromise in the methane yield suggesting that reduced methane yield with an increase in OL is primarily due to inhibitors present in the feed such as furans and soluble lignin. This implicates that Norway spruce hydrolysate pretreated at 140 °C is more suited for high rate reactors where only recalcitrant compounds and not inhibitors are influencing the methane yields, while for Norway spruce hydrolysate pretreated at 170 °C, both recalcitrant compounds and inhibitors are reducing methane rates and yields. At thermophilic condition, the effect of inhibitors was however more pronounced and influencing the methane yield from both Norway spruce hydrolysate pretreated at 140 °C and 170 °C.
- Norway spruce hydrolysate pretreated at 140 °C had higher methane yield during mesophilic condition compared to thermophilic condition suggesting pronounced effect of furan inhibitors during thermophilic condition. Although, the hydrolysate pretreated at 170 °C had lower methane yield during mesophilic condition, improved methane production rate could be observed during mesophilic AD of both hydrolysates.

5.3 Effect of pyrolysis temperature and biomass types on anaerobic digestion of aqueous pyrolysis liquid

- AD recovered reasonable amounts of carbon (32 to 59 %) from the APL of intermediate pyrolysis of Norway spruce and birch as biomethane in batch reactors, showing the potential of high carbon recovery from APL with well adapted microorganisms, improved continuous systems and enhancing additives.

- The biomethane yield in the case of Norway spruce APL was much lower (46 %) at pyrolysis temperature 550 °C compared to 400 °C while the methane yields of birch APL were about 50 % (based on COD) at both temperatures suggesting that the lower pyrolysis temperature is better for Norway spruce to produce APL with lower concentration of inhibitors from degradation of softwood lignin.
- A decrease in methane yield with increased carbon content in the APL was observed for both birch and Norway spruce. The effect is largest for Norway spruce (softwood) with a larger and less complex lignin content, leading to higher concentrations of recalcitrant and toxic compounds in the intermediate pyrolysis APL.

5.4 Effect of hot water extraction before pyrolysis on anaerobic digestion of aqueous pyrolysis liquid

- Mesophilic methane yield of the APL from pyrolysis of birch after HWE was higher than the APL from pyrolysis without HWE suggesting that HWE before pyrolysis is beneficial for birch if APL is considered as feed for AD as HWE improves sugar concentration (mainly levoglucosan) in APL while removing possible AD inhibitor (acetic acid, carboxylic acids, ketones and phenols) concentrations. However, the choice of tested HWE temperature had no significant effect on methane yield.
- HWE at 140 °C before pyrolysis decreased methane yield from APL of Norway spruce while HWE at 170 °C had no significant effect.
- Although the choice of HWE temperatures had no significant effect on methane yield, they had some effect on production rate in both Norway spruce and birch since HWE at 140 °C resulted in longer digestion time compared to 170 °C for

both biomass types suggesting higher reduction of possible inhibitor precursors at 170 °C.

5.5 Anaerobic co-digestion of hydrolysate and aqueous pyrolysis liquid

- Mesophilic co-digestion of APL with hydrolysate enhanced the overall methane yield at the COD based feed ratio of 3:1 (hydrolysate:APL) compared to the ratio of 1:1, while the enhancement was not significant compared to individual digestion suggesting that the use of APL as an additive feed in moderate ratios to hydrolysate in AD can be advantageous considering the volume of the feeds typically available in the process scheme investigated, the toxicity of the APL and the easy bio-degradation of the hydrolysate.

5.6 Further recommendations

5.6.1 Anaerobic digestion process

Most of the experiments performed during this study are batch tests to evaluate the biodegradability and methane production potential of the substrates. Thus, data obtained from the batch experiments such as methane yield can be used to study continuous tests which can be used to assess the performance and stability of reactors, adaptation and acclimatization of microorganism strains and breakdown of the inhibitors present in the hydrolysate and APL, which are more important for industrial application. Additives such as biochar, a solid by-product of the pyrolysis process, can also be used during the AD process to help the microorganisms cope with inhibitors in the hydrolysate and APL. Activated sludge from aerobic wastewater treatment plant can be utilized as co-substrate with hydrolysate and APL as a stable and nutritious source. Similarly, co-digestion with nitrogen rich substrate to maintain C/N ratio without nitrogen supplement (used in this study) is interesting to avoid extra cost. Syngas which is not considered in the integration during this PhD work should also be used as possible

AD feed to enhance energy recovery, perhaps in a pressure reactor for enhanced diffusion.

5.6.2 Pretreatment of anaerobic digestion feed

AD process is inhibited by various toxic compounds such as furans and phenolic present in the hydrolysate and APL. Therefore, detoxification strategies such as use of activated carbon, calcium hydroxide, ion exchange resins and oxidative process should be considered to lower negative impacts of toxic compounds on the methane production and cell growth.

5.6.3 Microbiology

Microbial communities should be studied to evaluate time needed for adaptation or alteration of microbial community to handle the inhibiting and toxic compounds present in the hydrolysate and APL.

5.6.4 Anaerobic digestion model 1 (ADM1) modeling

ADM1 modeling of continuous AD of hydrolysate with the consideration of inhibition of furfural and HMF is very useful for industrial application. Similarly, APL being a very challenging AD feed due to various known and unknown inhibitors, its simulation in ADM1 can give providing important information before considering it for pilot or industrial scale.

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Part II

Article 1

Thermophilic Methane Production from Hydrothermally Pretreated Norway spruce (Picea abies)

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Article

Thermophilic Methane Production from Hydrothermally Pretreated Norway Spruce (*Picea abies*)

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Abstract: Norway spruce (*Picea abies*) is an industrially important softwood species available in northern Europe and can be used to produce bio-methane after proper pretreatment to overcome its recalcitrant complex structure. Hot water extraction (HWE) pretreatment at two different conditions (170 °C for 90 min (severity 4.02) and 140 °C for 300 min (severity 3.65)) was applied to extract hemicellulosic sugars from Norway spruce for thermophilic anaerobic digestion (AD) of the hydrolysate. The methane yield of hydrolysate prepared at the lower pretreatment severity was found to be 189 NmL/gCOD compared to 162 NmL/gCOD after the higher pretreatment severity suggesting higher pretreatment severity hampers the methane yield due to the presence of inhibitors formed due to sugars and lignin degradation and soluble lignin, extracted partially along with hemicellulosic sugars. Synthetic hydrolysates simulating real hydrolysates (H170syn and H140syn) had improved methane yield of 285 NmL/gCOD and 295 NmL/gCOD, respectively in the absence of both the inhibitors and soluble lignin. An effect of organic loadings (OLs) on the methane yield was observed with a negative correlation between OL and methane yield. The maximum methane yield was 290 NmL/gCOD for hydrolysate pretreated at 140 °C compared to 195 NmL/gCOD for hydrolyate pretreated at 170 °C, both at the lowest OL of 6 gCOD/L. Therefore, both pretreatment conditions and OL need to be considered for efficient methane production from extracted hydrolysate. Such substrates can be utilized in continuous flow industrial AD with well-adapted cultures with stable organic loading rates.

Keywords: lignocellulosic biomass; Norway spruce; hot water extraction; hydrolysate; anaerobic digestion; thermophilic

1. Introduction

Biofuels are of great interest to reduce excessive dependence on fossil fuels that trigger issues related to global warming and energy security. Lignocellulosic biomass such as wood, food wastes, energy crops, and agricultural and forest residues are the most abundant renewable sources for biofuels and do not compete with food production. Methane production from such biomass can reduce a significant fraction of fossil fuel usage paving ways for a cleaner environment in a sustainable way.

Spruce, a softwood, is found abundantly as the major forest reserve in Nordic countries [1] and can be considered as a potential source of biogas due to its high carbohydrate content. However, its inherent recalcitrant structure and complex composition must be overcome to enhance hydrolysis and further conversion by anaerobic microorganism [2]. Various pretreatments, such as mechanical, thermal, chemical, and biological methods, have been attempted on lignocellulosic biomass prior to anaerobic digestion (AD) to enhance methane production. Hot water extraction (HWE) has several advantages and is widely accepted as a green technology as it involves only lignocellulosic biomass and water and avoids corrosion problems, acid recycling, and formation of neutralization sludges [3–5]. HWE, usually carried out at temperatures between 120 °C and 230 °C and various pressure conditions for different retention times, effectively dissolves hemicellulose sugars which may be beneficial to the anaerobic digestion in theory [6]. This liquid hydrolysate is rich in oligomeric and monomeric products like xylose, glucose, mannose, arabinose, and galactose [7] and is a suitable substrate for AD [8]. AD on hydrolysate, instead of AD on the original lignocellulose, overcomes problems related to traditional solid-state (SS) or semi SS-AD such as higher retention time, poor biodegradation, low methane yield, and acidification [9]. In addition to the hemicellulosic sugars in the liquid hydrolysate, inhibiting compounds are also formed due to degradation of sugar molecules during HWE [10] which usually inhibit bacteria and archea and operating parameters of the pretreatment need to be optimized to reduce their formation [11]. The nature and concentration of such inhibiting compounds depend upon the condition of pretreatment of the raw material types [12]. The main inhibitors are furfural and 5-hydroxymethylfurfural (HMF) formed by the dehydration of pentose and hexose sugars respectively in addition to degradation products of lignin polymer such as phenol, cresol, syringaldehyde, and vanillin [12].

Temperature plays a crucial role in AD as increased temperature leads to increased reaction rate in biochemical systems [13]. Thermophilic AD (55–60 °C) is considered a highly-efficient system due to a better pathogen inactivation and enhanced biogas production rate compared to mesophilic AD (35–40 °C) [13]. However, several studies have reported that thermophilic AD is susceptible to VFAs accumulation (especially propionic acid), inhibiting the methanogenic activity and decreasing the pH-buffer system [14–16]. Besides the operating condition, a higher proportion of feeding can also influence the rate of anaerobic digestion. The organic loading (OL) of the reactor with a reasonable amount of inoculum, also called substrate-to-inoculum ratio (S/I), is an important parameter when estimating methane potential [17]. High OL leads to VFA accumulation inhibiting the methanogens, thus lowering the amount of methane produced. On the contrary, lower OL cannot provide enough nutrition for microorganism growth, thus hampering the AD process [18].

Although hot water extracted hydrolysates of several agricultural residues and energy crops have been anaerobically digested to produce methane [11,19,20], AD of woody biomass hydrolysate (especially spruce) is still lacking. Among the available experiments, very few [21] are conducted in a thermophilic condition although it would save energy cost cooling the hydrolysates prepared at high temperatures. It is also imperative to test AD of hydrolysate prepared at different pretreatment conditions to have an overview of the effects of possible inhibitors in methane production due to the severity of the pretreatment.

This paper aims to evaluate the methane yield in thermophilic AD condition from hot water extract of Norway spruce pretreated at two different pretreatment severities, also by testing corresponding synthetic hydrolysates and the effect of organic loadings in AD.

2. Materials and Methods

Norway spruce is pretreated using hot water extraction before anaerobic digestion is conducted on the hydrolysate. Synthetic similar substrates, without inhibitors from the pretreatment, are also anaerobic digested and compared with the spruce hydrolysates. Two batch AD methods are used, syringe and automatic methane potential testing system (AMPTS II).

2.1. Raw Materials

Wood chips of Norway spruce was used for the experiment. The wood chips had a dry matter content of 44.5% when received from a Norwegian forestry company. The sample was air dried to 93.9% dry matter, hammer milled with 1000 RPM through a 19 mm hole screen and fractionated to a size between 13 mm and 5 mm.

2.2. Hot Water Extraction (HWE)

The wood chips were hot water extracted in a Mini-Mill Laboratory Digester (MMLD) from MK Systems Inc. Distilled water and wood chips were mixed in a 5:1 weight ratio and loaded into the MMLD before preheated at 110 °C for 20 min. The temperature was then increased to the target temperature of 140 °C or 170 °C over the course of 20 or 30 min, respectively, while the final temperature was kept for 300 min or 90 min, respectively. After the HWE, the hydrolysate was collected, analyzed and tested for anaerobic digestion.

In order to describe the combined effects of pretreatment time and temperature for each pretreatment, a severity factor (Equation (1)) [22] is calculated for each hydrolysate (Table 1). The temperatures and retention times were chosen based on literatures [10,23] so as to extract maximum hemicellulose from the biomass without the formation of AD inhibitors in the hydrolysate while also pretreating the solid residues to be used elsewhere such as bio-char production for enhanced carbon recovery. Pretreatment at 140 °C for 300 min and 170 °C for 90 min gives a severity factor (($\log(R_0)$) of 3.65 and 4.02 respectively, both considered as relatively moderate treatment severities with relatively low concentration of inhibitory degradation products [23].

$$\text{Severity factor} = \log(R_0) = \log\left(t \times \exp\left(\frac{T - 100}{14.75}\right)\right) \tag{1}$$

where T (°C) is the pretreatment temperature and t (min) is the reaction time.

Table 1. Severity factor for the hydrolysates from HWE.

Hydrolysate Samples	Hydrothermal Pretreatment Conditions Temperature (°C)	 Time (min)	Severity Factor ($\log(R_0)$)
H170	170	90	4.02
H140	140	300	3.65

2.3. Synthetic Hydrolysate

Synthetic hydrolysates H170syn and H140syn were prepared to closely simulate hydrolysate pretreated at 170 °C (H170) and 140 °C (H140) respectively, based on the concentrations of sugars and acetic acid and excluding the amount of furfural, 5-HMF, and soluble lignin (Table 2).

Table 2. Content of synthetic hydrolysates.

Parameters	H170syn	H140syn
Soluble COD (gCODs/L)	20.7	12.6
Arabinose (g/L)	0.81	1.63
Galactose (g/L)	2.17	1.67
Glucose (g/L)	3.00	1.55
Xylose (g/L)	2.24	1.95
Mannose (g/L)	10.39	5.11
Acetic acid (g/L)	1.03	0.59
pH	3.07	3.14

2.4. Anaerobic Digestion in Batch Reactors

The two hydrolysates from HWE of Norway spruce were tested for bio-methane potential (BMP) during syringe batch anaerobic digestion (AD) at different organic loads, while both hydrolysates and the synthetic hydrolysates are tested in the AMPTS II at one load.

All hydrolysates, including the synthetic, had micronutrients and macronutrients added. A macronutrient solution was made of NH_4Cl (44.48 g/L), $(NH_4)H_2PO_4$ (5.3 g/L), $(NH_4)_2HPO_4$ (1.78 g/L), $MgCl_2{\cdot}6H_2O$ (21.4 g/L), $CaCl_2{\cdot}2H_2O$ (7.56 g/L) and $NaHCO_3$ (100 g/L). Similarly, a micronutrient was prepared from the yeast extract (2.5 g/L), $FeCl_3{\cdot}6H_2O$ (0.2 g/L), $ZnCl_2$ (5.2 g/L), $MnCl_2{\cdot}4H_2O$ (0.0472 g/L), $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (0.064 g/L), $AlKO_8S_2{\cdot}12H_2O$ (0.01 g/L), $CoCl_2{\cdot}6H_2O$ (0.2 g/L), $NiCl_2{\cdot}6H_2O$ (0.52 g/L), H_3BO_3 (0.12 g/L), $CuCl_2{\cdot}2H_2O$ (0.32 g/L) and HCl (20 mL/L). The macronutrient solution was added to maintain a minimum COD:N:P ratio of 350:5:1 [24], while 4 mL micronutrient was added per 1000 L feed. Nutrients and NaOH addition increased the feed pH to 7.

2.5. Bio-Methane Potential AMPTS II Test Setup

The BMP test was performed [25] with the Automatic Methane Potential Test System II (AMPTS II, Bioprocess Control® AB, Lund, Sweden), a standardized laboratory set-up designed to automatically determine BMP of any biodegradable material by water displacement method. Each sample was run in triplicates (Table 3) in standard 650 mL glass flasks (Kimax® kimble) with working volume of 400 mL and the same organic loading of 20 gCOD/L. The system was purged with pure nitrogen gas for 3–5 min to ensure desired anaerobic condition. An intermittent mixing was 50 s every hour (motor speed adjustment of 80%). The carbon dioxide was removed by passing produced biogas through 80 mL of 3 M sodium hydroxide (NaOH) mixed with 0.4% thymolphthalein solution as pH-indicator for each reactor. The methane produced from AMPTS II were automatically provided as NmL by Bioprocess Control® software. A more detailed description of the system can be found in Badshah, Lam [26].

Table 3. Organic loading (OL) of AMPTS II-fed hydrolysates and synthetic hydrolysates.

Sample	Inoculum (mL)	Substrate (mL)	OL (gCODt/L)
H170	240	160	20
H140	200	200	20
H170syn	200	200	20
H140syn	160	240	20
Control (Blank)	240	160 (distilled water)	NA

NA: Not applicable.

2.6. Bio-Methane Potential Syringe Test Setup

Cheap disposable plastic medical syringes (BD Plastipak, Franklin Lakes, NJ, USA) of 100 mL were used as batch reactors for BMP test [27]. Triplicates of each OL of 6, 10, 20, and 30 g COD/L for both HWE feeds were prepared (Table 4) for bio-methane potential (BMP) tests to study the effect of OL on methane yield. After feeding, syringes were closed airtight by adding a needle with silicone rubber stopper at the tip. Fed syringes were kept at 55 °C in a thermostat. Stirring was not applied but frequent gas sampling acted as manual stirrer. During the test, gas volume was recorded depending upon the activity. After prolonged gas accumulation, gas (minimum 5 mL) was transferred to a separate syringe by pushing through interconnected gas valves (Mininert® syringe valve) for determination of methane content of the produced biogas. Methane volume was adjusted to 0 °C and 1 atm and presented as NmL.

Usually, OL is used interchangeably with substrate to inoculum (S/I) ratio. However, S/I is based on volatile solids (VS) of substrate and inoculum. The value of 0.5 (based on volatiles) has been found to be boundary to avoid overloading conditions if the inoculum is sewage sludge [28]. However, this value can change with the types of inoculum and substrate used. The S/I value can be higher if inoculum is compact such as granular sludge and lower if the substrate is more complex such as algae residues [29]. As our substrate is mostly liquid, COD based loading per volume of inoculum is more logical. In addition, volatiles such as acetic acid is lost during drying at 105 °C which gives incorrect methane potential values using VS.

Table 4. Organic loading (OL) of batch syringes fed HWE hydrolysates.

Sample	Inoculum (mL)	Substrate (mL)	OL (g CODt/L)
H170	15	3	6
H170	15	5	10
H170	15	10	20
H170	15	15	30
H140	15	4	6
H140	15	6.7	10
H140	15	13.4	20
H140	15	20	30
Control (Blank)	15	10 (distilled water)	NA

NA: Not applicable.

2.7. Inoculum

Mesophilic granular sludge used as inoculum was originally obtained from a mesophilic industrial internal recirculation reactor treating paper mill effluent with total solid and volatile solid concentrations of 181 and 119 g/L respectively. Inoculum was degassed at 30 °C for at least 5 days before using for the experiment to reduce the gas production from inoculum. Thermophilic sludge was prepared [30] by running initial similar tests in both syringes (Table 4) and AMPTS II (Table 3) at 55 °C for 53 days until complete methane production before used as inoculum in this experiments. Blank sample, only containing inoculum, was tested in triplicates along with the investigated samples. Gas produced from blank sample was deducted from the gas produced from hydrolysates to offset the gas produced by endogenous respiration of microorganisms in inoculum. The result thus represents gas produced only from the tested samples.

2.8. Analytical Methods

Gas composition was determined by SRI gas chromatography (model 8610C) (Table 5) using Helium as a carrier gas and the oven temperature was kept constant at 83 °C.

Table 5. Various analysis carried out during the experiment using different instruments.

Analysis	Instruments Used	References
Biogas composition	SRI gas chromatography (model 8610C)	[34]
VFA concentrations	Gas chromatography HP 6890 serial C (Hewlett Packard)	[34]
COD	Commercial kits (WTW™)	[31]
pH	WTW inolab pH7110	
Carbohydrate composition	Dionex ICS500 HPLC (ThermoFisher Scientific)	[32]
Furfural and HMF	UV 1800 from Shimadzu	[33]

VFA concentrations were carried out using gas chromatography HP 6890 serial C (Hewlett-Packard) with a flame ionization detector and a capillary column (DB-FFAP 30 m long and 0.25 mm ID, 0.25 µm film). Helium was used as the carrier gas at a flow velocity of 5 mL/min with detector gases as hydrogen and air. The injector and the detector temperatures were set to be 200 °C and 250 °C, respectively. The oven was programmed to start at 80 °C and hold for 1 min, then to 180 °C at a rate of 30 °C/min, then to 230 °C at a rate of 100 °C/min.

COD was measured according to US standard 5220D [31]. Samples were filtered through 0.45 µm pore size glass filter after sampling to measure CODs using commercial kits (WTW^{TM}). pH was measured using WTW inolab pH7110. The carbohydrate composition was analyzed according to the NREL procedure by Sluiter, Hames [32], using a Dionex ICS500 HPLC system from ThermoFisher

Scientific. Approximations of the furfural and HMF concentrations in the hydrolysates was done according to the procedure of Chi, Zhang [33], using a UV 1800 from Shimadzu.

2.9. Kinetic Modeling

Maximum methane production potential was determined by fitting the observed cumulative methane yield with the modified Gompertz equation Equation (2) [35]. It can simulate methane yield and explain the lag time and sigmoidal growth curve [36].

$$G(t) = G_0 \exp\left\{-\exp\left[\frac{R_{max} e}{G_0}(\lambda - t) + 1\right]\right\} \tag{2}$$

where G(t) is the cumulative methane production (mL CH_4 $gCOD^{-1}$) at a given time t, t is time over the digestion period in days (d), G_0 is the maximum methane production potential (mL CH_4 $gCOD^{-1}$), R_{max} is the maximal methane production rate (mL CH_4 $Gcod^{-1}$ day^{-1}), λ is the lag phase time in days (d), and e is Euler's constant (=2.7183).

3. Results

3.1. AD Feed Characteristics

Hot water extraction of Norway spruce led to higher organic concentrations in the hydrolysate when treated at 170 °C than at 140 °C, with total COD (CODt) and soluble COD (CODs) concentrations of 30.7 and 26.9 g/L respectively when treated at 170 °C compared to 22.3 and 20.0 g/L at 140 °C (Table 6).

Table 6. Characteristics of hydrolysate pretreated at 140 °C and 170 °C (Average values ± standard deviation (n, number of samples)).

Parameters	H170	H140
Total COD (gCODt/L)	30.7 ± 1.7 (41)	22.3 ± 1.6 (66)
Soluble COD (gCODs/L)	26.9 ± 2.6 (41)	20.0 ± 1.9 (66)
Acetic acid (g/L)	1.0 ± 0.2 (8)	0.6 ± 0.1 (14)
pH	3.7 ± 0.1 (2)	3.8 ± 0 (2)
Furfural (g/L)	0.9 ± 0.02 (2)	0.2 ± 0.01 (2)
5-Hydroxy methyl furfural (g/L)	0.5 ± 0.01 (2)	0.2 ± 0.01 (2)
Arabinose (g/L)	0.8 ± 0.003 (2)	1.6 ± 0.02 (2)
Galactose (g/L)	2.2 ± 0.01 (2)	1.7 ± 0.05 (2)
Glucose (g/L)	3.0 ± 0.01 (2)	1.6 ± 0.05 (2)
Xylose (g/L)	2.2 ± 0.02 (2)	2 ± 0.04 (2)
Mannose (g/L)	10.4 ± 0.02 (2)	5.1 ± 0.1 (2)
Total sugars (g/L)	18.6 ± 0.01 (2)	11.9 ± 0.3 (2)

The sugars found in the hydrolysate were the hemicellulose sugars arabinose, galactose, glucose, xylose, and mannose. Mannose was the major compound in both hydrolysates with 56% and 43% for pretreated at 170 °C and 140 °C, respectively. The total sugar concentration was higher (18.6 g/L) when treated at 170 °C than at 140 °C (11.9 g/L). All other individual sugar concentrations, except arabinose, was found higher in hydrolysate pretreated at 170 °C than at 140 °C.

Both furfural and HMF concentrations were higher in hydrolysate pretreated at 170 °C (923 and 451 mg/L respectively) than pretreated at 140 °C (160 and 168 mg/L respectively) due to higher severity pretreatment.

The pH of hydrolysate pretreated at 170 °C (3.7) was lower than for hydrolysate pretreated at 140 °C (3.8) due to higher acetic acid released from the hydrolysis of acetyl groups contained in the hemicellulose. Acetic acid was the dominant volatile fatty acid in the hydrolysate and is also a substrate for methane production if the concentration is not above the threshold value of inhibition

(2400 mg/L) [37,38]. The concentrations were below for both hydrolysates with acetic acid concentration of hydrolysates pretreated at 170 °C of 1030 mg/L and at 140 °C of 590 mg/L.

3.2. AMPTS II Test

Methane yield in AMPTS II was recorded for 8 days in which methane formation was completed within 6 days and resulted in cumulative methane yields of 189 NmL/gCOD (0.53 gCOD/gCOD) and 162 NmL/gCOD (0.45 gCOD/gCOD) for hydrolysate pretreated at 140 °C and 170 °C, respectively (Table 7).

Table 7. Initial pH values and effluent characteristics at the end of AMPTS II experiments.

Samples	Initial pH	End pH	End CODs (mg/L)	End Acetic Acid (mg/L)	End Propionic Acid (mg/L)	End Total VFA (mg/L)	Methane Yield (gCOD/gCOD)
H170	7	7.97 ± 0.06	5780 ± 200	97 ± 22	112 ± 30	221 ± 42	0.45 ± 0.01
H140	7	8.00 ± 0.04	4730 ± 30	67 ± 8	36 ± 7	103 ± 6	0.53 ± 0
H170syn	7	7.77 ± 0.05	2490 ± 120	15 ± 4	0	15 ± 4	0.84 ± 0.01
H140syn	7	7.73 ± 0.05	1970 ± 120	15 ± 7	0	15 ± 7	0.84 ± 0.01

The methane yield of real hydrolysates was significantly lower than of synthetic hydrolysates (Table 7). Synthetic hydrolysate had 87% and 58% higher methane yield than hydrolysate prepared at 170 °C and 140 °C, respectively. Values of CODs and accumulated VFA (acetic acid, propionic acid, and total) (Table 7) at the end of the experiment shows higher degradability of synthetic hydrolysates compared to real hydrolysates as observed by lower concentration of undigested CODs and VFA. Synthetic hydrolysates had negligible amount of VFA which is in accordance with its high biodegradability while hydrolysate pretreated at 170 °C had more than double total VFA concentration (221 mg/L) compared to hydrolysate pretreated at 140 °C (103 mg/L). Even propionic concentration (112 mg/L) was higher than acetic acid (97 mg/L).

3.3. Kinetic Modeling

The modified Gompertz model was applied for modelling the methane yield of both real and synthetic hydrolysates from the AMPTS II tests (Figure 1). It shows that the tested model provided reasonable fit to the experimental data (Table 8). It was confirmed by the high values (all above 0.96) of determination coefficient (R^2) and less than 4% difference between the predicted (G_0) and the measured cumulative methane yield. It implies that the model could explain greater than 96% of the variations in the results.

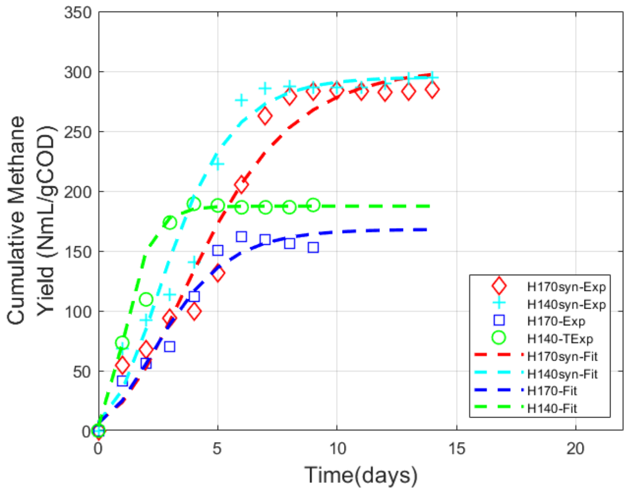

Figure 1. Cumulative methane yield of hydrolysate pretreated at 140 °C and 170 °C from AMPTS II fitted with modified Gompertz law.

Table 8. Regression results of cumulative biogas yield with the modified Gompertz model.

Samples	G_0 (NmL CH_4 gCOD $^{-1}$)	R_{max} (NmL CH_4 Gcod $^{-1}$Day^{-1})	λ (Days)	R^2	Cumulative Methane Yield (NmL gCOD^{-1})
H170	168.0	34.0	0.4	0.962	162
H140	187.4	99.8	0.3	0.962	189
H170syn	302.9	42.2	0.8	0.960	285
H140syn	295.2	60.1	0.6	0.960	295

Hydrolysate pretreated at 170 °C had a lower maximum methane production rate (R_{max} = 34 mL CH_4 gCOD^{-1}day^{-1}) compared to hydrolysate pretreated at 140 °C (R_{max} = 100 mL CH_4 gCOD^{-1}day^{-1}). It shows that higher severity index pretreatment not only reduced the methane yield, but also decreased the rate of the AD process.

The lag phases (Table 8) during the beginning of the digestion period were less than 0.4 days for all substrates. Interestingly, the synthetic hydrolysates had slightly higher lag phase in both cases and lower R_{max} in case of 140 °C hydrolysate compared to real hydrolysates for some unknown reasons.

3.4. Syringe Tests

The batch experiments in syringes were run for 38 days until the biogas production became negligible but most of the biogas was produced before 10 days.

3.4.1. Influence of Organic Loading on AD of Hydrolysate

Increasing OL of the hydrolysate pretreated at 170 °C had negative effect on the methane yield (Figure 2). Methane yields at OL of 6 and 10 gCOD/L were 0.56 gCOD/gCOD (195 NmL/gCOD) and 0.53 gCOD/gCOD (186 NmL/gCOD) respectively. At higher loadings of 20 and 30 gCOD/L, the methane yield decreased even more to 0.48 (167 NmL/gCOD) and 0.43 gCOD/gCOD (152 NmL/gCOD) respectively.

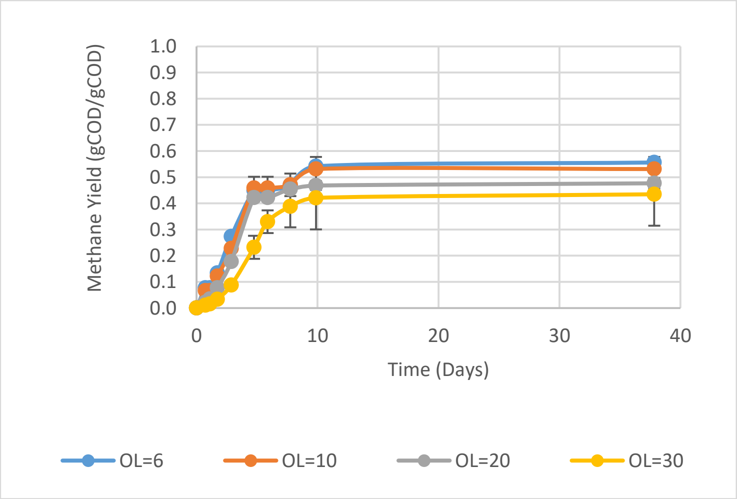

Figure 2. Methane yield of H170 under thermophilic condition under different organic loadings (OLs).

The methane yields from hydrolysate pretreated at 140 °C (Figure 3) had a similar trend as that of hydrolysate pretreated at 170 °C. During the lower OLs of 6 and 10 gCOD/L, the methane yield was 0.83 (290 NmL/gCOD) and 0.80 gCOD/gCOD (282 NmL/gCOD) respectively while decreasing to 0.71 (247 NmL/gCOD) and 0.6 gCOD/gCOD (211 NmL/gCOD) for 20 and 30 gCOD/L respectively. The methane yield of hydrolysate pretreated at 140 °C was significantly higher than hydrolysate pretreated at 170 °C at all OLs (Figure 4), as also observed during AD in AMPTS II.

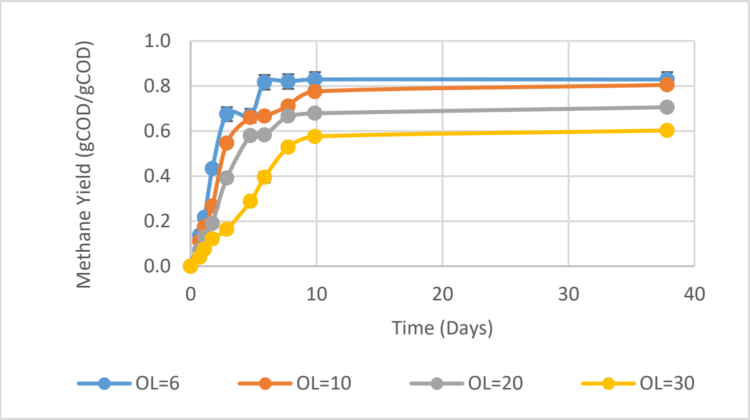

Figure 3. Methane yield of H140 under thermophilic condition under different OLs.

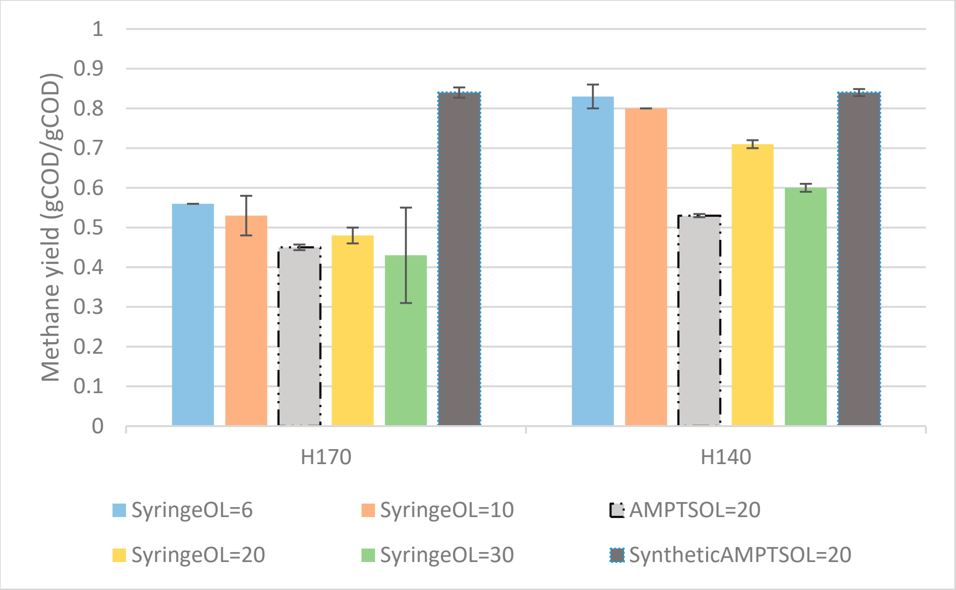

Figure 4. Methane yield in syringes for both hydrolysates at varying OLs. Yields from AMPTS II are added for comparison.

3.4.2. pH and Methane Content in the Biogas

The methane content, ranging from 10% to 84 %, increased over time, and remained constant at the later stages for both substrates at all loadings (Figures 5 and 6). The main difference between the substrates was the higher methane content of hydrolysate pretreated at 140 °C than 170 °C during the initial period of higher loadings suggesting higher inhibitor concentration in higher severity pretreated hydrolysate. The low initial biogas methane content also implies that the methanogenesis step was inhibited. The final weighted-average methane content in the biogas ranged from 64.9% to 73.3% (Table 9). It was higher for hydrolysate pretreated at 170 °C compared to 140 °C and decreased at increased OL.

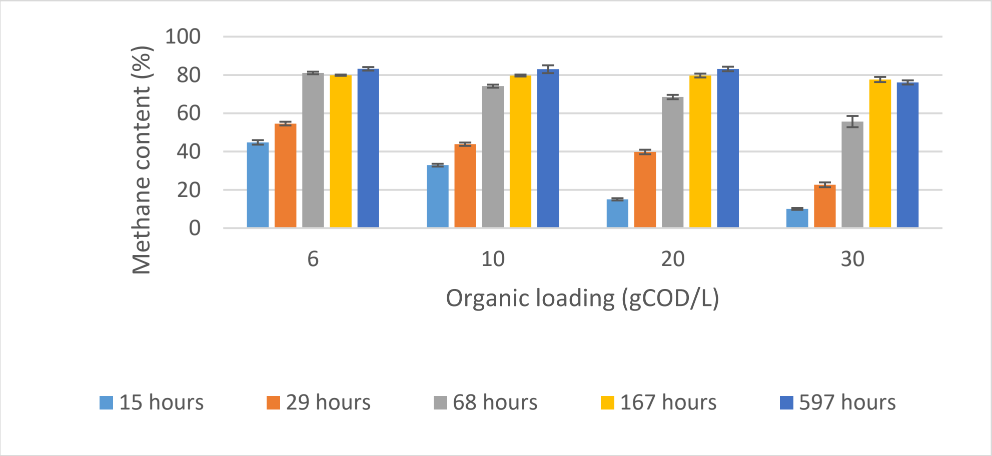

Figure 5. Methane content of H170 at different OLs over time in syringes.

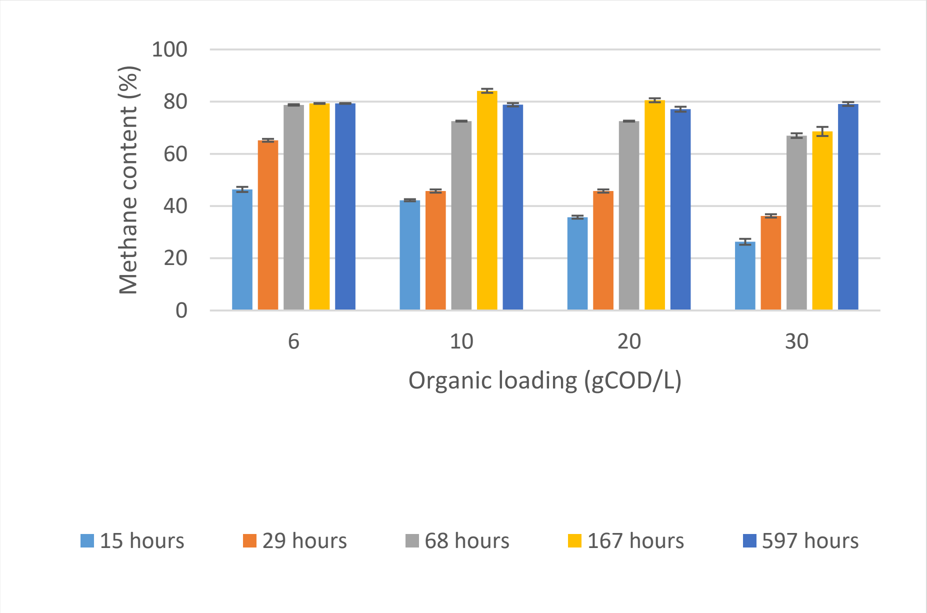

Figure 6. Methane content of H140 at different OLs over time in syringes.

Table 9. Weighted average methane content in the produced biogas in syringes.

OL (gCOD/L)	H170	H140
6	73.3 ± 0.3	70.1 ± 0.4
10	69.4 ± 0.4	65.7 ± 0.1
20	69.5 ± 0.8	66.4 ± 0.3
30	68.2 ± 1.4	64.9 ± 0.8

4. Discussion

4.1. Effect of Sugars

Hydrolysate pretreated at 170 °C had a higher concentration of sugars and COD values compared to hydrolysate pretreated at 140 °C due to increased solubilization of macromolecular organic compounds (e.g., hemicelluloses) that hydrolyzed into small molecular matter like oligosaccharides and monosaccharide (e.g., glucose and xylose). A similar trend has been observed by others [39,40]. Higher methane yield for hydrolysate produced at 140 °C than at 170 °C is also observed by others [11,36]. Contrary to different studies, that higher sugar content in the hydrolysate is best for methane production [20], it shows that it is not necessarily true. The reason is assumed to be the formation of sugar and lignin degradation products (e.g., furfural, HMF, phenol, cresol) which inhibit the AD process [2,19]. Therefore, sugar content should not be the sole objective to be considered if methane production is the main goal, and the amounts of inhibitors and soluble lignin in the hydrolysate needs to be considered [19,41]. The severity index during the pretreatment appears to be a useful control parameter to obtain fewer inhibitors, solubilize lignin and cellulose in the hydrolysate and to obtain a solid residue ideal for other purposes, like making bio-char.

Usually, glucose is present in the hydrolysate due to hydrolysis of cellulose but as the HWE is not severe enough to cause dissolution of cellulose, it is expected to have originated from hemicelluloses or extractives [6,42].

4.2. Effect of Sugar Degradation Products

Higher pretreatment severity leads to higher sugar dissolution which increases the concentration of sugar degradation products such as furfural and HMF which inhibit the AD process [2]. Furfural is formed in the hydrolysate as degradation product from pentose sugars (xylose and arabinose) while HMF is formed from hexose sugars (mannose, glucose, and galactose) [43]. Increase in severity leads to low arabinose concentration in hydrolysate pretreated at 170 °C due to its assumed conversion to furfural. Since only arabinose and xylose can be converted to furfural as they are pentose sugars, and arabinose has the lowest activation energy in the degradation reaction of arabinose to furfural when compared to that of xylose to furfural a conversion of arabinose is assumed [19].

Although the concentration of furfural and HMF is within the threshold value (2 g/L for furfural and 0.8 g/L for HMF) for both the samples [44], inhibition could be expected from the synergistic effect of both inhibitors [45]. Although inhibitors are also considered to be more pronounced at thermophilic condition than mesophilic condition [46,47], this may not be a problem in continuous flow industrial processes since the microorganisms can get acclimatized to toxic compounds over time. The inhibitors are themselves degraded in an adapted culture, but if furfural is present in the HMF, the conversion rates of both decrease significantly and HMF degradation started only with the complete degradation of furfural in batch experiments [48]. Continuous flow experiments are needed to better understand the effects of hydrolysate inhibitors on AD and culture adaptations to such.

4.3. Effect of Soluble Lignin and its Derivatives

During hot water extraction, part of the lignin is also dissolved along with the hemicellulose depending upon the severity index [4,7]. Hydrolysate from higher pretreatment temperature is therefore expected to have higher concentrations of complex recalcitrant compounds and soluble lignin than lower temperature pretreated hydrolysate. These compounds will remain undigested, are slowly degraded, or act as inhibitors to different steps of AD [41,49,50] and can be observed as higher undigested CODs at the end of the experiment (Table 7). Decrease in methane yield has been observed previously due to the addition of lignin during AD of xylose [12].

Although not quantified here, it can be safely assumed that there must be some lignin degradation products such as phenols and cresols due to long residence time in HWE which inhibit the AD process [51,52].

4.4. Effect of OL

Methane yield of hydrolysate pretreated at 140 °C at OL of 6 gCOD/L was similar to that of synthetic hydrolysate (Figure 4) suggesting concentration of inhibitors below threshold values and favorable organic loading for AD microbes. This is similar to findings of wheat straw hydrolysate [8], but no similar studies on woody biomass hydrolysates are found for comparison. Additionally, most of the existing experiments on hydrolysate from agricultural residues are based on mesophilic conditions.

The trend that the comparative cumulative methane yield (gCOD/gCOD) from the syringe batch reactors decreased with increased OLs ($R^2 = 0.995$ for both hydrolysates) is also observed by others [18] and can be explained by increased stress on microorganisms. Lower OL provides favorable microbial symbiosis leading to higher methane yield while higher OL leads to imbalance between VFA production and its consumption.

4.5. Methane Content as a Tool to Monitor Reactor Health

Monitoring the methane content to evaluate possible overloading situations revealed a rather normal behavior [18,53] where the methane content in the produced biogas increased over time and remained nearly constant at the later stages for both substrates at all loadings. The behavior can be explained by VFA production and consumption affecting the pH. Soluble sugars are the most readily biodegradable organics and are converted into VFAs and finally to methane [20]. Easily degraded substrate can lead to accumulation of intermediate products such as alcohol and VFAs due to imbalance of micro-organisms created by stressful conditions such as nutrient deficiency and toxicity in the feed, leading to thermodynamic and kinetic constraints in the system. Then microorganisms are unable to convert acetate to methane efficiently, compromising the methane content [19]. Overtime, VFA is consumed slowly increasing pH, creating more favorable conditions for methanogens, and the methane concentration increases accordingly. During lower loading, the initial condition is not as acidic compared to higher loading avoiding pH inhibition of the methane production. The initial pH drop due to excessive VFA accumulation and methanogens inhibition increases with OL as confirmed by low methane concentration (Figure 6) [37]. The reactor recovered from this initial stress in our cases (end pH between 8.1 and 8.3) with reasonable final methane concentrations in all cases (Table 9). This implies that continuous flow industrial AD will work well on these substrates as long as large, abrupt organic load changes are avoided.

4.6. Kinetic Modeling

170 °C synthetic hydrolysate also had lower R_{max} compared to 140 °C synthetic hydrolysate but the difference was not as drastic as in real hydrolysates and can be attributed to the higher concentration of xylose in 170 °C synthetic hydrolysate which is generally less preferred to degrade as feed by microorganisms than other sugars [54]. A short lag phase of less than 0.4 days was also observed by others during AD of sugars [55].

4.7. Comparison of AD Batch Methods

Comparing the methane yields from hydrolysate pretreated at 170 °C and 140 °C for both real hydrolysates and synthetic hydrolysates at 20 gCOD/L, the AMPTS II gave lower methane yields compared to the syringe test method (Figure 4), and has also been reported lower than the German DIN standard method using eudiometers [56]. Hydrolysate pretreated at 170 °C had 7% higher methane yield in the syringe method (0.48 gCOD/gCOD) versus AMPTS II (0.45 gCOD/gCOD), while hydrolysate pretreated at 140 °C had 34% higher in syringe method (0.71 gCOD/gCOD) versus AMPTS II (0.53 gCOD/gCOD). The difference can be attributed to human error due to manual operation [57], while headspace gas concentration in syringe and microbiology of AD also can be affected due to change of temperature while removing incubated syringes from the temperature-controlled environment during measurement of gas [58]. The relative differences between the different feeds compared are similar for the two methods implying that both methods worked well for the comparisons in this study.

5. Conclusions

Hydrolysates of Norway spruce from hot water extract are a promising feed for anaerobic digestion and hydrothermal pretreatment conditions influence both methane production rates and yields. Higher pretreatment severity yielded higher concentrations of AD inhibitors. Despite having lower sugar content, hydrolysate pretreated at the lower temperature of 140 °C had 18% higher methane yield (0.53 gCOD/gCOD) than higher pretreatment temperature of 170 °C (0.45 gCOD/gCOD). Comparison of methane yield between real hydrolysate and synthetic hydrolysate showed that soluble lignin and inhibitors (furans) had a significant effect on methane yield. Synthetic hydrolysate had 87% and 58% higher methane yields than hydrolysates prepared at 170 °C and 140 °C, respectively. Negative correlation was observed between methane yield and organic loadings (OLs) of the hydrolysates prepared at both temperatures. The higher organic loadings were found to cause the most stress initially, assumed to be mainly due to inhibition of the methane production, but all batch reactors eventually recovered with reasonably high final methane yields. This implies that these substrates can be utilized safely in continuous flow industrial AD with well adapted cultures where large, abrupt organic load changes are avoided.

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Article 2

Mesophilic Anaerobic Digestion of Hydrothermally Pretreated Lignocellulosic Biomass (Norway Spruce (Picea abies))

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Article

Mesophilic Anaerobic Digestion of Hydrothermally Pretreated Lignocellulosic Biomass (Norway Spruce (*Picea abies*))

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Abstract: Hot water extraction (HWE) removes hemicellulose from woody biomass to give improved end products while producing a sugar-rich by-product stream, which requires proper treatment before disposal. Hot water extracted Norway spruce (*Picea abies*) at two different pretreatment conditions (140 °C for 300 min (H140) and 170 °C for 90 min (H170)) generated hydrolysate as a by-product, which was used in mesophilic anaerobic digestion (AD) as substrate. H140 gave a higher methane yield (210 NmL/g COD—chemical oxygen demand) than H170 (148 NmL/g COD) despite having a lower concentration of sugars, suggesting that different levels of inhibitors (furans and soluble lignin) and recalcitrant compounds (soluble lignin) affected the methane yield significantly. Organic loads (OLs) had a negative effect on the methane yield, as observed during AD of H170, while such an effect was not observed in the case of H140. This suggests that the decrease in methane yield (32%) of H170 compared to H140 is primarily due to inhibitors, while the decrease in methane yield (19%) of H140 compared to the synthetic hydrolysate is primarily due to recalcitrant substances. Therefore, both OL and pretreatment conditions must be considered for efficient anaerobic digestion from hydrolysate for enhanced methane production.

Keywords: mesophilic anaerobic digestion; hydrolysate; lignocellulosic biomass; hot water extraction; Norway spruce

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1. Introduction

Lignocellulosic woody biomass is used for various products, such as wood composites, board products, and biochar [1,2]. Different pretreatment methods, such as mechanical, chemical, biological, physiochemical, and hydrothermal, are in use to make woody biomass more suitable for end products [1]. Among these methods, HWE is a common hydrothermal pretreatment carried out in the temperature range 120–230 °C and pressure conditions at which water is kept in subcritical condition [3]. As it is a chemical-free and environmental-friendly process, its use in lignocellulosic pretreatment is increasing [4], mainly to extract hemicellulose. During HWE, water molecules penetrate the lignocellulose network to solubilize hemicellulose sugars, such as xylose, glucose, mannose, arabinose, and galactose, and slightly remove lignin [5,6]. Removing the hemicellulose makes the wood rich in cellulose and lignin, which has several positive effects important for wood composites and various board products [1]. Pyrolysis of the wood after HWE also gives better bio-oil yield, reduces the content of ketones, acids, and water in the bio-oil leading to higher heating value and significant improvement of levoglucosan content [7], improving the quality of the bio-oil produced by pyrolysis [8–10]. The hemicellulosic stream is the by-product sugar-rich liquid, commonly called hydrolysate, and requires proper treatment before discarding to avoid environmental deterioration. Since the hydrolysate is rich in pentose sugar, it is not considered suitable for bioethanol production, due to the requirement of genetically modified microorganisms to degrade pentose, and these are highly sensitive to furan inhibitors like furfural and hydroxyl methyl furfural (5-HMF) present in the hydrolysate [11,12]. It has also been considered for the production of hydrogen through dark fermentation, but without promising results [13,14].

A suitable alternative for utilizing the hydrolysate can be anaerobic digestion (AD), a robust and mature technology consisting of a consortia of naturally occurring microorganisms that can adapt to synergistically break down various complex, recalcitrant, and even inhibitory compounds to methane [15]. A wide range of organic materials can, therefore, be used as feedstock in AD to produce biogas after proper pretreatment [16]. Moreover, liquefaction of lignocellulosic biomass overcomes several obstacles, such as acidification, low methane yield, poor biodegradation, and long retention time, which are inherent in traditional solid-state (SS) or semi-SS AD [17]. Biogas, a renewable and clean biofuel, is produced by AD of organic substances and is the most abundant gaseous biofuel. Feeding proportion can also influence the AD rate. Organic loads (OLs, or feed to microorganism ratio F/M) influences AD efficiency and needs to be optimized for industrial application [18]. Higher organic loading rates (OLR) can lead to the accumulation of volatile fatty acids (VFA), inhibiting AD [19]. Lower OLRs are also not beneficial for efficient industrial applications.

As co-production of biochar and bio-oil has increased in recent years to replace fossil-based fuels [20], hydrolysate formed as a by-product during pretreatment needs to be handled well to enhance the carbon recovery. Various lignocellulosic hydrolysate of agricultural by-products and energy crops, such as sugarcane bagasse, wheat, Napier grass, rice straw, etc., have been considered for AD [5,21,22]. Although hydrolysate of woody biomass, such as Norway spruce, has been used recently in thermophilic AD conditions for methane production [23], its use in mesophilic AD is yet to be exploited.

The goal of this research is to study the effects of pretreatment severities on the mesophilic AD methane yield of the hot water extracted hydrolysates of Norway spruce. Effects of hydrolysate inhibitors and recalcitrant substances on yield are investigated by testing corresponding synthetic hydrolysates, without inhibitors from the pretreatment, and the effects of OL in AD. The automatic methane potential testing system (AMPTS II) and syringe methods are used as batch AD.

2. Materials and Methods

2.1. Hot Water Extraction (HWE) Producing Hydrolysates

Norway spruce wood chips, with a dry matter content of 44.5%, were received from a Norwegian pulp and paper mill (Norske Skog Saugbrugs AS, Halden). They were predried (93.9% DM—dry matter) and fractionated to a size between 13 mm and 5 mm, and mixed with distilled water in a 5:1 weight ratio. The mixture was loaded into a preheated Mini-Mill Laboratory Digester (MMLD, M/K Systems Inc., Williamstown, Massachusetts, USA) at 110 °C for HWE of wood chips and treated for 20 min at this temperature before the target temperature of 140 °C or 170 °C was reached over the course of 20 or 30 min, respectively. The target temperatures were kept for 300 min or 90 min, respectively [23]. The hydrolysates were collected and analyzed after HWE before testing for AD.

A severity factor (Equation (1)) [24] describing the effect of pretreatment time and temperature combined is calculated for each hydrolysate. Chosen temperatures and retention time values were from available references [25,26] to maximize hemicellulose extraction from the wood chips, while reducing AD inhibitor concentrations in the hydrolysate. Pretreatment at 140 °C for 300 min (H140) and 170 °C for 90 min (H170) gives moderate severity factors ($\log(R_0)$) of 3.65 and 4.02, respectively, producing hydrolysates with relatively low concentration degradation products inhibiting the AD process [26].

$$\text{Severity factor } (\log(R_0)) = \log\left(t \times \exp\left(\frac{T - 100}{14.75}\right)\right) \tag{1}$$

where, t (min) is the reaction time, and T (°C) is the pretreatment temperature.

2.2. Synthetic Hydrolysate

The real hydrolysates H140 and H170 were closely simulated by preparing synthetic hydrolysates H140syn and H170syn, respectively, based on the sugars and acetic acid concentrations, while excluding the inhibitors, such as furfural, 5-HMF, and soluble lignin (Table 1), resulting in soluble chemical oxygen demand (COD) values for H140syn and H170syn of 12.6 and 20.7 g CODs/L, respectively.

Table 1. Content of synthetic hydrolysate. (chemical oxygen demand) COD, chemical oxygen demand.

Parameters	H140syn	H170syn
Soluble COD (g CODs/L)	12.6	20.7
Arabinose (g/L)	1.63	0.81
Galactose (g/L)	1.67	2.17
Glucose (g/L)	1.55	3.00
Xylose (g/L)	1.95	2.24
Mannose (g/L)	5.11	10.39
Acetic acid (g/L)	0.59	1.03
pH	3.14	3.07

2.3. AD Batch Reactors Feeding

Both Norway spruce hydrolysates and the synthetic hydrolysates were tested for methane production in the AMPTS II at one OL, while both real hydrolysates were tested during AD in syringe batch reactors at different OLs.

All hydrolysates, also the synthetic, were added micro- and macronutrients [23] where a minimum COD:N:P ratio of 350:5:1 [27] was maintained.

The inoculum used in the experiments was mesophilic granular sludge originally obtained from a reactor treating paper mill effluent with a density of 1.0 to 1.09 kg/m^3 and a diameter of 0.6 to 2.7 mm. The inoculum had total and volatile solid concentrations of 181 and 119 g/L, respectively. Possible gas production from the inoculum was reduced by degassing at 30 °C for >5 days before using in the experiment. The as produced from the blank sample, only containing inoculum and run in triplicates, was subtracted from the gas produced from the reactors to give gas production value only from the hydrolysates.

2.4. Methane Potential Test in AMPTS II

The automatic methane potential test system II (AMPTS II, Bioprocess Control® Sweden AB, Lund, Sweden, 2017), a standardized laboratory set-up, was used for the methane production test. It is used to determine methane production from any biodegradable material. Three parallels were run for each sample (Table 2). The experimental procedures can be found in Ghimire et al. [23]. The methane production values from AMPTS II were recorded as NmL (1 standard atmospheric pressure, 0 °C and zero moisture content) by Bioprocess Control® software. Badshah et al. can be referred for a detailed description of the AMPTS II system [28].

Table 2. Organic load (OL) of hydrolysates (real and synthetic) in an automatic methane potential testing system (AMPTS II) test.

Sample	Inoculum (mL)	Substrate (mL)	OL (g CODt/L)	Parallels
H140	200	200	20	3
H170	240	160	20	3
H140syn	160	240	20	3
H170syn	200	200	20	3
Control (Blank)	240	160 (DW)	-	3

DW, distilled water.

2.5. Methane Potential Test in Syringe

Real hydrolysates were run in triplicates for each OL of 6, 10, 20, and 30 g COD/L (Table 3) in 100 mL plastic medical syringes as batch reactors for methane production [29]. The detailed procedure can be found in Ghimire et al. [23]. Methane volume was adjusted to 1 atm, zero moisture content and 0 °C, and presented as NmL.

Table 3. OL of real hydrolysates in batch syringes.

Sample	Inoculum (mL)	Substrate (mL)	OL (g CODt/L)	Parallels
H140	15	4	6	3
H140	15	6.7	10	3
H140	15	13.4	20	3
H140	15	20	30	3
H170	15	3	6	3
H170	15	5	10	3
H170	15	10	20	3
H170	15	15	30	3
Control (Blank)	15	10 (DW)	-	3

DW, distilled water.

2.6. Analytical Methods

VFA concentrations, biogas composition, COD, pH, carbohydrate composition, furfural, and HMF are measured as described in Ghimire, Bakke, and Bergland [23].

2.7. Kinetic Modeling

The observed cumulative methane yield was fitted with the modified Gompertz model (Equation (2)) to determine the maximum methane production potential [30]. Methane yield with a sigmoidal growth curve, and the lag phase is simulated with the model [31].

$$G(t) = G_0 \exp\left\{-\exp\left[\frac{R_{max} e}{G_0}(\lambda - t) + 1\right]\right\} \tag{2}$$

where, *G* (*t*) is the cumulative methane production (NmL CH_4 /g COD) at a given time t, G_0 is the maximum methane production potential (NmL CH_4 /g COD), t is time over the digestion period in days (d), λ is the lag phase time in days (d), R_{max} is the maximal methane production rate (NmL CH_4/(g COD d)), and e equals 2.7183 (Euler's constant).

2.8. Statistical Analyses

One-way analysis of variance (ANOVA) was used for statistical analyses using MS-Excel 2016 (Microsoft Corporation, Redmond, Washington, US, 2020). $P < 0.05$ was denoted as statistical significance.

3. Results

3.1. Characteristics of Hydrolysate

HWE hydrolysate properties were dependent on pretreatment severity. Total and soluble COD values of H170 were higher (31 and 27 g CODt/L, respectively) compared to the values of H140 (22 and 20 g CODs/L, respectively). Similarly, higher acetic acid concentration in H170 (1.0 g/L) than H140 (0.6 g/L) gave a slightly lower pH value of 3.7 compared to 3.8 of H140. Total sugar concentration was also higher in H170 (18.61g/L) than H140 (11.9 g/L). Higher sugar concentration also leads to higher concentrations of furfural (0.9 g/L) and HMF (0.5 g/L) in H170 compared to 0.2 g/L of both furfural and HMF in H140. Detailed analysis of sugars can be found elsewhere [23].

3.2. AMPTS II Test

Maximum cumulative methane yields of 0.60 g COD/g COD (210 NmL/g COD) and 0.41 g COD/g COD (148 NmL/g COD) were observed for H140 and H170, respectively, compared to 0.74 g COD/g COD and 0.72 g COD/g COD of H140syn and H170syn, respectively (Table 4). A long phase of no gas production was observed after initial methane yields of 86 NmL/g COD and 73 NmL/g COD for H140syn and H170syn, respectively. Methane production recovered after 13 days and reached the final methane yield of 260 NmL/g COD (0.74 ± 0.01 g COD/g COD) and 252 NmL/g COD (0.72 ± 0.02 g COD/g COD) for H140syn and H170syn, respectively.

The methane yields of H140 and H170 were 0.19 g COD/g COD and 0.43 g COD/g COD lower (significant, $p < 0.05$) than the methane yields of their synthetic hydrolysate equivalents. The difference (significant, $p < 0.05$) between H140 and H170 was 0.32 g COD/g COD. The end pH was higher in synthetic hydrolysates than real hydrolysates, well above initial pH in all the cases, suggesting no overloading conditions in the end.

Table 4. Initial pH values, end AMPTS II liquid characteristics, and methane yield. VFA, volatile fatty acids.

Samples	**Initial pH**	**End pH**	**Initial CODs mg/L (Feed Only)**	**End CODs mg/L (Feed and Inoculum)**	**End Acetic Acid (mg/L)**	**End Propionic Acid (mg/L)**	**End Total VFA (mg/L)**	**Methane Yield (g COD/g COD)**
H140	6.1	6.9 ± 0.0	20000	1933 ± 63	ND	ND	ND	0.60 ± 0.02
H170	5.9	7.0 ± 0.1	20000	1723 ± 62	ND	ND	ND	0.41 ± 0.00
H140syn	6.1	7.6 ± 0.1	20000	1670 ± 216	ND	ND	ND	0.74 ± 0.01
H170syn	6.0	7.7 ± 0.1	20000	2313 ± 68	ND	ND	ND	0.72 ± 0.02

ND, not detected.

3.3. Kinetic Modeling

The G_0 values predicted by the model are similar to the experimental cumulative methane yield (Figure 1) for the real hydrolysates, while a temporary production stop influenced the synthetic hydrolysate experimental cumulative methane yield curve. The difference between the predicted and the measured methane yield was less than 4% in the case of real hydrolysates, while less than 12% in the case of synthetic hydrolysates.

The lag phase was found to be lowest for H140 (0.04 d) compared to H170 (0.44 d). H140 had similar R_{max} (79 NmL CH_4/(g COD d)) to H170 (78 NmL CH_4/(g COD d)) (Table 5).

H140syn (13 NmL CH_4/(g COD d)) and H170syn (12 NmL CH_4/(g COD d)) also had equal R_{max}. The low rates calculated based on the Gompertz model (Equation (2)) are, however, not very appropriate for such cases where there is a pause instead of a lag phase in gas production. The R_{max} measured directly (from data in Figure 1) is similar to the R_{max} of the real hydrolysates.

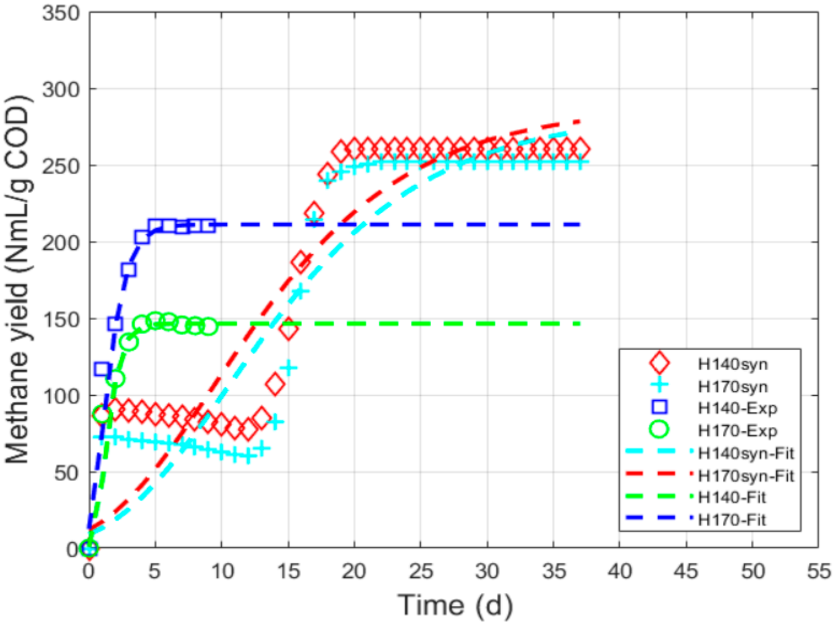

Figure 1. Modified Gompertz model (Equation (2)) fitted with cumulative methane yield of hydrolysates (real and synthetic) from AMPTS II. COD = chemical oxygen demand

Table 5. Model parameters by the modified Gompertz model from cumulative methane production.

Samples	G_0 (NmL CH_4/g COD)	R_{max} (NmL CH_4/(g COD d))	λ (d)	R^2	Cumulative Methane Yield (NmL/g COD)
H140	211	79	0.04	0.958	210
H170	146	78	0.44	0.931	148
H140syn	288	13	1.27	0.860	260
H170syn	284	12	1.99	0.863	252

3.4. Syringe Tests

3.4.1. OL Influence on AD of Hydrolysate

The batch experiments in syringes fed H140 and H170 at different OLs were run for 103 days (Figures 2–4). The biogas production started immediately at the low OLs for both H140 and H170, implying that the culture was ready to digest the sugars and acetic acid present in the hydrolysates. Increasing OL beyond 10 g COD/L had a significant effect on the methane yield of H170, unlike H140 that had a slightly lower methane production rate, but without a compromise in the methane yield. Methane yields of 0.79 g COD/g COD and 0.74 g COD/g COD were obtained at the low OLs of 6 and 10 g COD/L, respectively, for H170, while yield values decreased to 0.57 g COD/g COD and 0.54 g COD/g COD for OLs of 20 and 30 g COD/L, respectively. H140 had methane yield near 0.7 g COD/g COD) for all OL. A long lag phase was observed at the highest load (30 g COD/L) in H170 (25 d), unlike in H140, which had no lag phase, but a slower digestion rate at the highest OL.

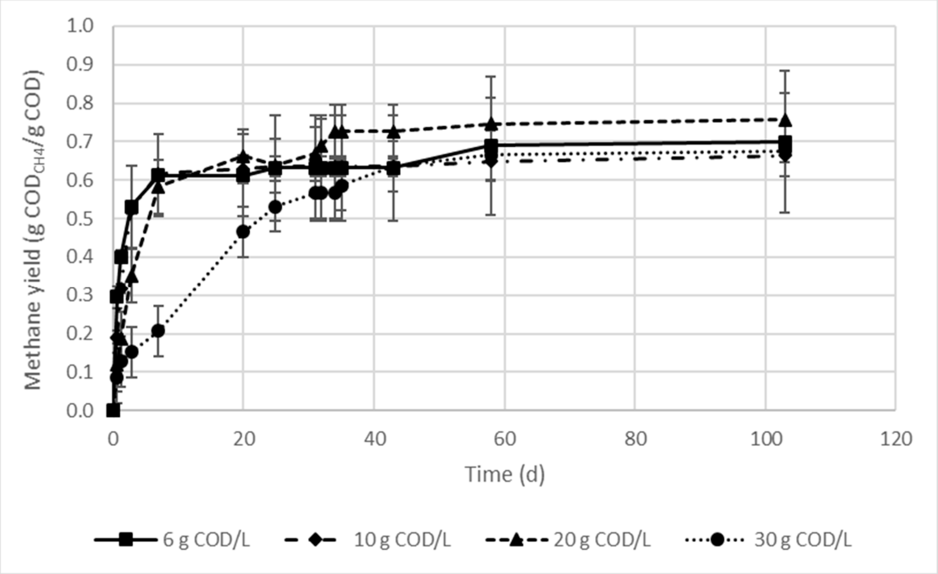

Figure 2. Methane yield of H140 at mesophilic conditions under different organic load (OL). COD = chemical oxygen demand.

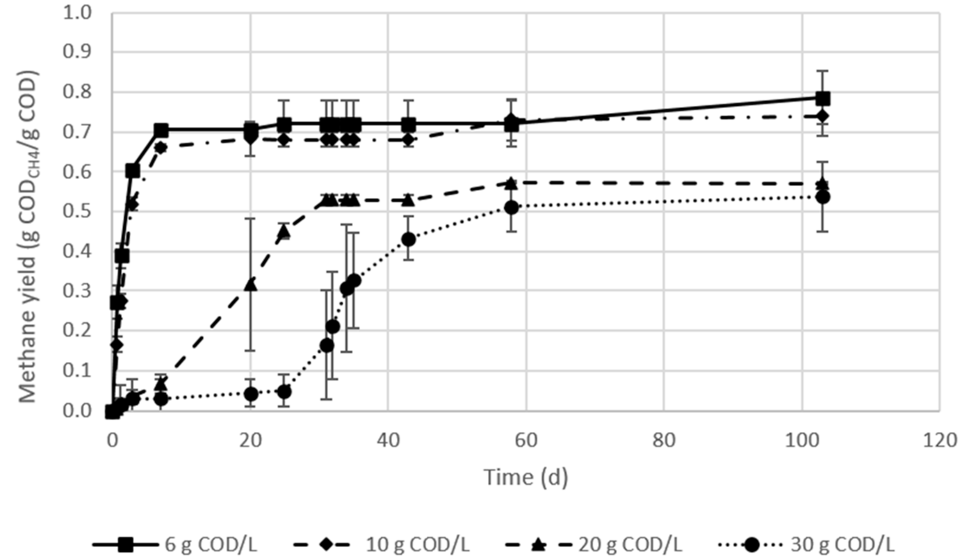

Figure 3. Methane yield of H170 at mesophilic conditions under different organic load (OL). COD = chemical oxygen demand.

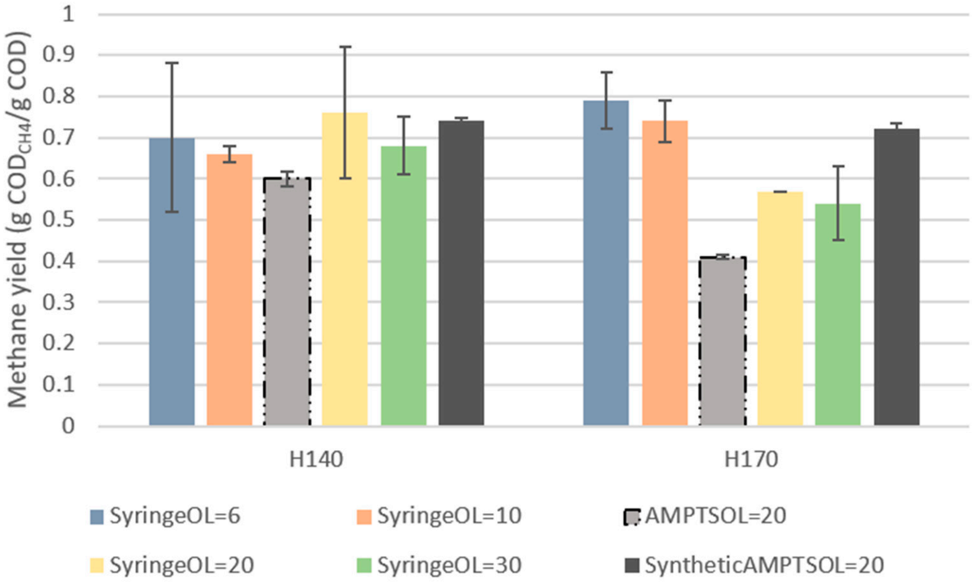

Figure 4. Methane yield of H140 and H170 at different organic loads (OLs) in syringes. Yields from AMPTS II is also added for comparison. COD = chemical oxygen demand.

3.4.2. Methane Content and pH

The methane content of both substrates either increased with time or remained constant towards the end at all loads, and the value ranged from 2 to 86% (Figures 5 and 6). The main difference between the substrates was the higher methane content of H140 than H170 during the initial period of higher loads. During the loading of 20 g COD/L, H140 had methane content of 32%, 46%, and 60% compared to only 2%, 10%, and 39% of H170 at 15, 29, and 68 h, respectively. Similarly, during the loading of 30 g COD/L, H140 had methane content of 31%, 28%, and 63% compared to only 4%, 5%, and 32% of H170 at 15, 29, and 167 h, respectively. The difference decreased with time, all ending up with near 80% final methane content (Figures 5 and 6, and Table 6). The weighted average methane contents ranged from 58% to 77% (Table 6). The weighted average methane content increased with an increase in OLs and was higher for H170 compared to H140.

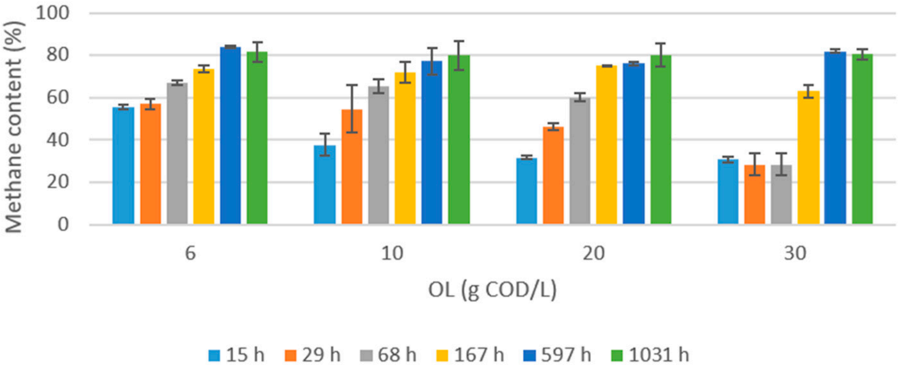

Figure 5. Methane content in biogas produced at different organic loads (OLs) over time in syringes H140. COD = chemical oxygen demand.

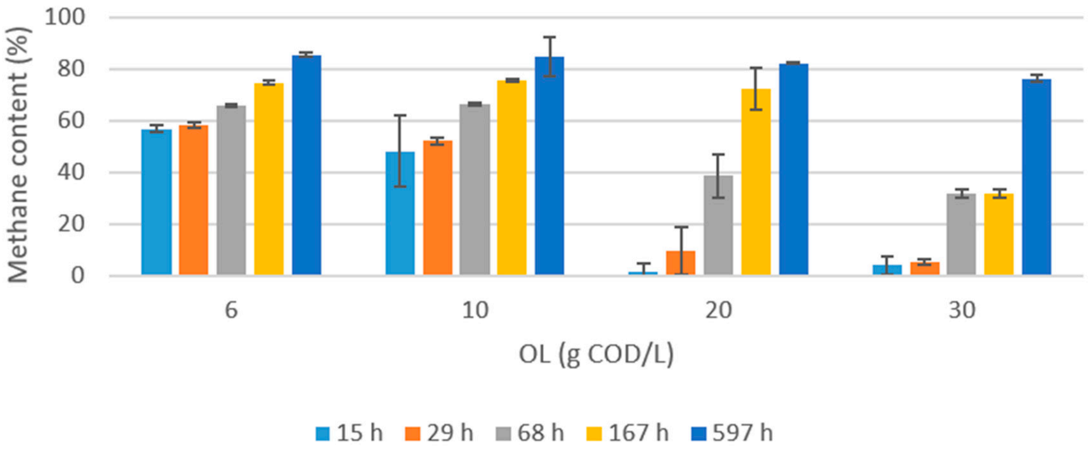

Figure 6. Methane content in biogas produced at varying organic loads (OLs) with time in syringes fed H170. COD = chemical oxygen demand.

Table 6. Weighted average and final methane content of biogas in syringes.

OL (g COD/L)	Weighted Average H140	Final H140	Weighted Average H170	Final H170
6	63.6 ± 0.8	83.9 ± 0.6	64.7 ± 0.5	85.4 ± 0.7
10	57.8 ± 5.5	80.0 ± 6.9	63.8 ± 3.8	84.6 ± 7.5
20	64.5 ± 1.1	80.1 ± 5.4	76.5 ± 1	82.2 ± 2.1
30	65.3 ± 1.7	80.5 ± 2.4	72.9 ± 1.0	76.1 ± 2.5

4. Discussion

4.1. Effect of Sugars

The methane yield of hydrolysates from Norway spruce is similar to hydrolysate from agricultural residues, such as sugarcane bagasse and sunflower oil cake [32,33]. Comparing methane yield of hydrolysate from Norway spruce used in this experiment with thermophilic AD condition [23] shows a similar trend with a higher methane yield of H140 than of H170. The methane yields of the synthetic hydrolysates were slightly lower than the observed values under thermophilic conditions [23]. It is not clear why, but a long pause in gas production indicates some overloading conditions where an intermediate product may have inhibited methanogenesis.

Despite having a higher sugar concentration, H170 did not produce more methane than H140, so higher concentrations of COD and sugars do not necessarily lead to higher methane yields [5]. Inhibitory soluble lignin and sugar degradation products in the hydrolysate need to be considered along with sugar content if AD is the main aim [5,34]. The temperature and retention time during HWE can be tuned to enhance hemicellulose solubilization, while limiting the formation of sugar degradation products, lignin, and cellulose extraction and solubilizing, thereby obtaining more solid residues for biochar production, more bio-oil production, and hydrolysate suitable for methane production.

4.2. Effect of Sugar Degradation Products

Concentrations of AD inhibiting sugar degradation products, such as HMF and furfural, increase with higher pretreatment severity [35], and H170 had a higher concentration of inhibitors than H140. Since the concentration of sugar degradation products are less than the threshold values (0.8 mg/L for HMF and 2 g/L for furfural) that AD can tolerate, the lower methane yield of H170 can be attributed to the synergistic effect of both inhibitors, hampering microbial activities [36]. Inhibition was pronounced at higher OLs for H170, but not so for the H140, consistent with Monlau et al. [35] that inhibitor concentrations increase with temperature. The inhibitors themselves can be degraded during

AD, but if HMF is present with furfural, the degradation rates of both decrease significantly, and HMF degradation starts after the complete furfural degradation [37]. However, microorganisms can get adapted to inhibitors over time, and the effects of inhibitors in hydrolysate on AD and adaptation of inoculum microorganisms should be better studied in long-term continuous flow experiments.

4.3. *Effect of Soluble Lignin and Its Derivatives*

Depending upon the severity index, part of the lignin can be dissolved during HWE along with the hemicellulose [6,38], so H170 is expected to have a higher concentration of lignin than H140, due to higher pretreatment severity [25]. The use of this hemicellulose concentrated hydrolysate as AD substrate is mainly hindered by the presence of the lignin polymers and oligomers [39]. Soluble lignin is inhibitory to different steps in AD, with methane yield decreasing with increased lignin addition during AD of cellulose and hemicellulose [40]. The soluble lignin itself remains undigested or is very slowly degraded in AD [34,41,42]. This is consistent with the observed lower methane yields (Figure 3), despite having a higher concentration of sugars, probably due to higher concentrations of soluble lignin in H170 compared to H140.

Accumulated VFA (acetic acid, propionic acid, and total), pH, and CODs at the end of the experiment had similar values in both the real and the synthetic hydrolysates. However, the methane yield is lower in the case of real hydrolysates compared to synthetic hydrolysate (Table 5), while having similar CODs removal values. This can probably be due to the accumulation of undigested lignin, for instance, by adsorption on the granules [43]. The residual CODs can be attributed to recalcitrant compounds present in the inoculum (blank), which had values of 1753 ± 403 mg/L at the end of the experiment.

Generally, due to the possible formation of phenolic compounds from lignin degradation at temperatures above 200 °C, such is not considered for hydrothermal pretreatment (HTP) in this investigation. Although H170 was a reasonable temperature, a longer retention time increased its severity index to 4.02, which is comparable to pretreatments at 190 °C for 20 min, 200 °C for 10 min, and 210 °C for 5 min. Although phenolic compounds were not quantified in our sample, it can be assumed that such compounds were present and may have enhanced the before mentioned inhibition causing lower methane yields from H170 at higher loads.

4.4. *Effect of OL*

H140 is better suited for higher loads compared to H170 (Figure 4), which is consistent with results from thermophilic AD [23]. Slower kinetics at higher loads for both hydrolysates can be attributed to inhibition from temporary increased VFA concentrations. The slower kinetics is most pronounced for H170, which is therefore attributed to higher inhibitor concentrations (furans and possible soluble lignin). The methane yield is also lower, but the high methane concentration and low VFA concentration, in the end, suggest that this is not primarily due to an imbalance between VFA production and consumption, but rather the inhibitors present in the feed. Since high load did not compromise methane yields for H140 where the methane content reached high levels faster (Figure 5) and the inhibitor concentrations were low, it suggests that: (1) Lower methane yield (19%) during the AMPTS II AD of H140 compared to the synthetic hydrolysate is primarily due to recalcitrant compounds and not inhibition; (2) while the lower methane yield (32%) of H170 compared to H140 is primarily due to inhibitors.

4.5. *Kinetic Modeling*

The experimental data curve fitted with modified Gompertz equation shows that the experimental data fitted reasonably with the tested model with a determination coefficient (R^2) above 0.93 for both H140 and H170, which implies that the model could explain 93%

of the variations in the results of the hydrolysates, a value in agreement with hydrothermal pretreatment of rice straw being utilized for AD [31]. Low values (less than 0.5 d) of lag phase suggest the easy biodegradation of hydrolysates because of a high-sugar content and lack of lipids and proteins. Higher severity index had no significant effect on R_{max}, unlike G_0.

4.6. Comparison of AD Batch Methods

Compared to syringe tests, AMPTS II gave lower methane yield values (Figure 4) for both H140 and H170, and has also been reported lower than thermophilic syringe tests [23] and German DIN standard method using eudiometers [44]. H170 had 28% lower methane yield in AMPTS II (0.41 g COD/g COD) versus the syringe method (0.57 g COD/g COD), while H140 had 21% lower methane yield in AMPTS II (0.60 g COD/g COD) versus the method (0.76 g COD/g COD). The differences can be attributed to temperature change in the reactor when incubated syringes are removed from the temperature-controlled heat cabinet, due to manual operating that influenced AD microbiology and the headspace gas concentration in syringe [45], and human error due to manual operation [46]. In this study, different feeds compared showed similar relative differences suggesting that both the methods worked well for the comparisons.

5. Conclusions

HWE is an efficient pretreatment method to extract hydrolysate from Norway spruce to use as feed for AD. However, methane production yields and rates are influenced by pretreatment conditions. H140 gave a higher methane yield (210 NmL/g COD, 0.60 g COD/g COD) than H170 (148 NmL/g COD, 0.41 g COD/g COD) despite having a lower concentration of sugars, suggesting that different levels of inhibitors (furans and soluble lignin) and recalcitrant compounds (soluble lignin) had a significant effect on the methane yield.

A negative effect of OLs on methane yield was observed during AD of H170, while such an effect was not observed in the case of H140. This suggests that the decrease in methane yield (32%) of H170 compared to H140 is primarily due to inhibitors, while the decrease in methane yield (19%) of H140 compared to the synthetic hydrolysate is primarily due to recalcitrant substances. Therefore, both OL and pretreatment conditions must be considered for efficient anaerobic digestion of hydrolysate for enhanced methane production.

Abbreviations

AD	Anaerobic Digestion
AMPTS	Automatic Methane Potential Testing System
COD	Chemical Oxygen Demand
DM	Dry Matter
F/M	Feed to Microorganism Ratio
H140	Hydrolysate Pretreated at 140 °C
H140syn	Synthetic feed simulating H140
H170	Hydrolysate Pretreated at 170 °C
H170syn	Synthetic feed simulating H170
HMF	Hydroxymethyl Furfural
HWE	Hot Water Extraction
MMLD	Mini-Mill Laboratory Digester
OL	Organic Load
OLR	Organic Loading Rate
SS AD	Solid-State Anaerobic Digestion
VFA	Volatile Fatty Acid

Author Contributions: Conceptualization, N.G. and W.H.B.; methodology, N.G.; validation, N.G.; formal analysis, N.G. and W.H.B.; investigation, N.G. and W.H.B.; data curation, N.G.; writing—original draft preparation, N.G.; writing—review and editing, N.G., W.H.B. and R.B.; supervision, W.H.B. and R.B.; funding acquisition, R.B. All authors have read and agreed to the published version of the manuscript.

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Article 3

Improved Carbon Balance for Biocarbon Production by combination of Pyrolysis and Anaerobic Digestion

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Improved carbon balance for biocarbon production by combination of pyrolysis and anaerobic digestion

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Solid carbon is an important raw material in industrial processes. Most of today's charcoal is produced by conventional carbonisation which suffers from huge carbon losses due to system inefficiency. Intermediate pyrolysis is principally similar to conventional carbonisation and produces biocarbon while capturing the off gasses, among these the aqueous condensate which is difficult to utilise due to the high water content and low energy content. This fraction can contain up to 25 % of the carbon from feedstock, so utilisation of this fraction is important for good overall carbon balance. Anaerobic digestion can be a promising tool for utilising the carbon in the aqueous condensate by converting it to biomethane. Here, birch and spruce have been pyrolyzed and the biomethane potential for the aqueous condensates has been tested. Mass and carbon balances of the pyrolysis of birch and spruce at two pyrolysis temperatures were performed, and biocarbon carbon yields ranging from 42 % to 54 % were obtained. Anaerobic digestion of the aqueous phases from pyrolysis was performed with carbon recovery yields between 44 % and 59 %. A total carbon recovery of 77.8 % to 85.7 % was obtained and the main carbon losses have been identified.

Keywords: Biocarbon; Pyrolyis-anaerobic digestion combination; Increased carbon yield;

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INTRODUCTION

In order to reduce consumption of fossil resources, efforts should be made in providing new and renewable alternatives. The metallurgical industry requires a carbon material to act as a reducing agent and energy source, and a transition from fossil coal to renewable carbon would give a huge reduction in global fossil CO_2 emissions. Although many industrial processes already use charcoal, the majority of today's charcoal is still produced in traditional kilns (Bailis et al., 2013), e.g. earth mound kiln in sub Saharan Africa and "hot tail" kilns in Brazil (Pennise et al., 2001). Usually these traditional kilns do not have off gas utilisation or recovery, resulting in large emissions of incomplete combustion products into the atmosphere, which has a larger global warming impact than the molar CO_2 equivalent of complete combustion of the off gasses (Bailis, 2009). In addition, these emissions are harmful for humans and can increase mortality and respiratory diseases for populations close by points of emission (Bailis et al., 2005).

Brazil is the largest charcoal producer in the world and the main type of kilns used are "hot tail" kilns (Bailis et al., 2013). Although more efficient than most earth-mound kilns used in sub-Saharan Africa, the "hot tail" kilns have a reported maximum charcoal mass yield of 34.1 % and a charcoal carbon yield of 52.1 % (Pennise et al., 2001). This results in about 65 % and 50 % loss in mass and carbon, respectively, due to system inefficiency by venting of off gasses.

Modern pyrolysis, thermal decomposition without oxygen, is a relatively simple yet powerful primary conversion technique and is fundamentally similar to charcoal production. Pyrolysis can process a large variety of feedstocks and has been regarded as a promising route for biomass utilization for a long time (Maschio et al., 1992), but has struggled to find commercial feasibility. During the last decades, the research focus of biomass pyrolysis has predominantly been on fast pyrolysis with the aim of optimizing the bio-oil yield and quality (Bridgwater, 2012). In recent years, emphasis on co-production of bio-oil and biocarbon has increased (Laird et al., 2009) as numerous applications and considerable environmental benefits of biocarbon have been recognized (Cha et al., 2016).

Intermediate pyrolysis is a relatively new genre within pyrolysis which balances the biocarbon and bio-oil yields. Typically, 30 wt. % biocarbon is obtained with intermediate pyrolysis, which is in the upper range of traditional charcoal kiln, as opposed to 12 wt. % biocarbon with fast pyrolysis. The increased biocarbon yield is a result of the decrease in heating rates and increased reaction time compared to fast pyrolysis. Fast pyrolysis processes its feedstock within seconds, intermediate pyrolysis is usually complete within 30 to 90 minutes, while the “hot tail” kilns have a reported run time of 40 to 50 hours (Pennise et al., 2001).

The bio-oil from intermediate pyrolysis usually phase separates into an organic condensate phase and an aqueous condensate phase, most likely due to secondary cracking of the vapours before condensation (Yang et al., 2014). This improves the viscosity and heating value of the organic condensate compared to fast pyrolysis oil and can be used as an energy carrier. The aqueous condensate, however, contains a considerable amount of water and water-soluble components, has low calorific value and no direct area of application. This condensate fraction can still contain up to 25 % of the carbon of the feedstock, thus it is important to utilise this fraction to ensure efficient carbon utilisation and prevent discharge of polluted water. A promising route for utilisation of the carbon in the aqueous condensate of intermediate pyrolysis (also called aqueous pyrolysis liquid (APL)) is biomethane production by anaerobic digestion (Fabbri & Torri, 2016; Feng & Lin, 2017; Hübner & Mumme, 2015).

Anaerobic digestion (AD) is a biological process in which consortia of microorganisms break down organic compounds to produce biogas (typically consisting of 50-75% CH_4 and 25-50% CO_2) in the absence of free oxygen. It is a mature, well-established and robust technology in which mixed communities of organisms synergistically break down various easily degradable organics but can also digest more complex, recalcitrant and inhibiting compounds in low concentrations after some adaption time (Benjamin et al., 1984; Vasco-Correa et al., 2018). Biogas production by anaerobic digestion of organic wastes is regarded as an effective waste treatment and an energy production technology (Appels et al., 2008; Khalid et al., 2011). However, anaerobic digestion of raw lignocellulosic biomass has been proven difficult due to the recalcitrant nature of lignocellulosic biomass (Yang et al., 2015). It is nevertheless a promising technique for carbon recovery from aqueous side streams, like APL from intermediate pyrolysis. Although APL is a complex substrate with hundreds of compounds and a few compounds toxic to the AD microorganisms, they are able to adapt to a wide range of chemical substance which can be exploited to overcome the complexity of APL (Torri & Fabbri, 2014). Moreover, production of biomethane is a clean energy source that can be used as drop-in fuel after purification. This can be lucrative and is already available as a viable alternative as transportation fuel (Appels et al., 2011).

Research on AD of APL is still in its infancy, but increasing with the increased research focus of pyrolysis as a measure to handle the aqueous side stream (Feng & Lin, 2017; Hübner & Mumme, 2015). The application of APL in other fields has been hampered because of its low calorific value, acidity, chemical and thermal instability and presence of complex and inhibitory compounds (Kan et al., 2017; Zhou et al., 2019). AD of APL from pyrolysis of agricultural residues are examined to some extent, but little research has been done with wood as feedstock where the pyrolysis process is focused on the biochar production and quality.

The purpose of this work is to compare the carbon utilisation of commercial charcoal production with biocarbon production by intermediate pyrolysis combined with anaerobic digestion. The "hot tail" kiln process reported by Pennise et al. (2001) is chosen to be the benchmark process for commercial charcoal production since it is the most common charcoal production method in Brazil. Pyrolysis of two feedstocks at two temperatures have been performed along with the biomethane potential studies of the corresponding aqueous condensates.

EXPERIMENTAL

Raw materials

Woodchips made from Norway Spruce and Birch were used for this experiment. Norway Spruce chips were received from a Norwegian forestry company, with a dry matter content of 44.5 %. The sample was air dried to 93.9 %, hammer milled with 1000 RPM through a 19 mm hole screen and fractionated to a size between 13 mm and 5 mm. Birch chips were received dry from a Norwegian sludge refining company, with a dry matter content of 93.4 %. The birch chips were fractionated to a size between 13 mm and 5 mm.

Fig. 1. Prepared raw materials before pyrolysis. a) Birch. b) Spruce.

Pyrolysis

The wood chips were pyrolyzed in a pyrolysis development unit at RISE PFI AS, see Figure 2. The pyrolysis development unit consists of a stainless-steel fix bed reactor of 5.6 L located in an oven. A heated gas transfer line connects the reactor with a condensation unit, consisting of two water-cooling condensers, an electrostatic precipitator (ESP) and a silica gel filter. The gas volume was measured continuously with a wet gas meter (WGM) and a micro-GC (Agilent Technology 490 Micro GC) measured the gas composition online. N_2 gas was used as purge gas during the entire experiment with a flow of 2 l/min.

Typically, the reactor was filled with 400 g feedstock and leak tested before the entire system was purged with N_2 to remove oxygen. An oxygen free atmosphere was confirmed with micro-GC before starting the experiment. Heating time from room temperature to the two reaction temperatures of 400 °C and 550 °C was approximately 45 minutes. The reaction temperature was maintained for 90 minutes to ensure complete and homogeneous carbonisation of the feedstock. This resulted in 4 biocarbon samples, 4 condensate samples and 4 sets of gas phase analysis. The vapours condensed in the water condenser naturally phase separated into an organic condensate phase and an aqueous condensate phase. The condensate was stored in the condensate bottles overnight before the top phase was decanted off.

The silica gel filter before the μ-GC is required to obtain a moisture free gas for μ-GC analyses. The silica gel turns black instead of white during operation, suggesting that more than moisture is absorbed in the silica gel filter. This is most likely bio-oil mist not condensed in the preceding condensers. Analysis of the absorbate of the silica gel filter is not possible, so it was assumed that the composition is similar to the aqueous condensate and included in the aqueous condensate in the results.

Water content was measured in all condensate phases with Karl-Fischer titration on a Mettler Toledo V20 Volumetric KF Titrator. Carbon content of the feed, biocarbon and condensate phases was analysed on a Thermo Scientific Flash 2000 CHNS/O Analyzer. The aqueous condensates produced were used for anaerobic digestion.

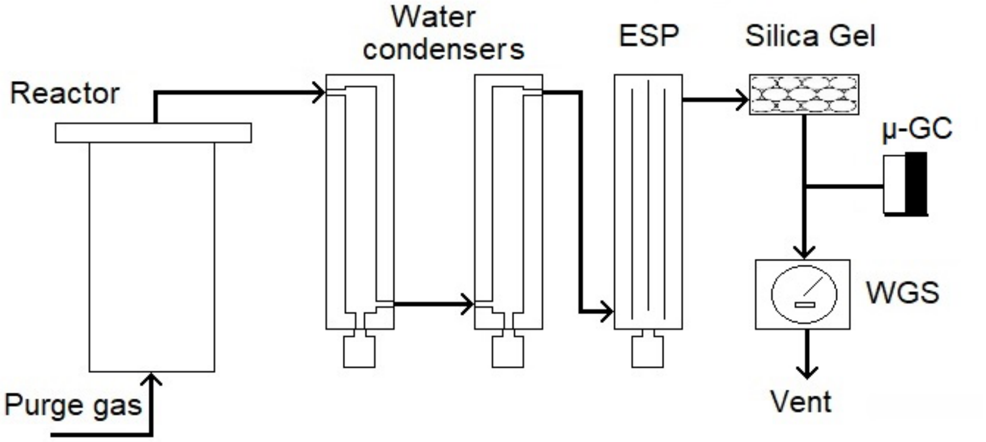

Fig. 2. Schematic of the setup of the pyrolysis rig as used for the pyrolysis experiments at RISE PFI.

Anaerobic digestion

Anaerobic digestion (AD) of APL as a substrate was performed as a batch test with the Automatic Methane Potential Test System II (AMPTS II, Bioprocess Control® Sweden AB). This standardized laboratory set-up automatically determines methane potential of any biodegradable material by the water displacement method.

Mesophilic granular sludge used as inoculum was obtained from a mesophilic industrial AD reactor treating paper mill effluent. Concentration of inoculum total solids (TS) and volatile solids (VS) was 181 g/L and 119 g/L respectively. The inoculum was degassed at 30 ºC for at least 5 days before the experiment to reduce the gas production from the inoculum.

The APL substrate was added a macro nutrient solution composed of NH_4Cl (44.48 g/L), $(NH_4)H_2PO_4$ (5.3 g/L), $(NH_4)_2HPO_4$ (1.78 g/L), $MgCl_2.6H_2O$ (21.4 g/L), $CaCl_2.2H_2O$ (7.56 g/L) and $NaHCO_3$ (100 g/L). The macro nutrient solution was added to the substrate to maintain a minimum COD:N:P ratio of 350:5:1 (Baeta et al., 2013).

Each APL sample was run in triplicates (Table 1) with equal APL load in standard 650 mL glass flasks (Kimax® kimble) added 200 mL inoculum creating a working liquid volume of slightly more than 200 mL.

Blank sample, only containing inoculum and distilled water, was also tested in triplicates. Gas produced from blank sample was deducted from the gas produced from APL samples to offset the gas produced by endogenous respiration of microorganisms in inoculum. The result thus represents only the gas produced from the tested samples.

The anaerobic reactors were purged with pure nitrogen for 5-7 min. initially to remove oxygen and ensure complete anaerobic condition. AD was performed at 35°C for 22 days with intermittent mixing of 10 seconds every hour. The carbon dioxide content in the produced biogas was removed by passing it through 80 mL of 3 M sodium hydroxide (NaOH) mixed with 0.4 % thymolphthalein solution for each reactor. The methane produced from AMPTS II were automatically provided as NmL (1 standard atmospheric pressure, 0 °C and zero moisture content) by Bioprocess Control® software. A more detailed description of this procedure is found in Badshah et al. (2012).

Table 1: Experimental design for AD of APL

Samples	Inoculum (mL)	APL substrate (mL)	Parallels	Organic Loading (gCOD APL/$L_{inoculum}$)
Birch, 400°C	200	1.4	3	5
Birch, 550°C	200	1.8	3	5
Spruce, 400°C	200	2.3	3	5
Spruce, 550°C	200	2.3	3	5
Control	200	3 (dist. H_2O)	3	0

Chemical oxygen demand (COD) was measured according to US standard 5220D (APHA, 1995). Samples were filtered through 0.45 µm pore size glass filter after sampling to measure soluble COD (sCOD) using commercial kits (WTW™).

RESULTS AND DISCUSSION

The effect of feedstock and pyrolysis temperature on carbon yield

The mass balances of the birch and spruce chips pyrolyzed at 400 °C and 550 °C are given in Figure 3. The mass recovery of biocarbon, condensates and gas phase add up to about 100 % for each experiment, as intended by the design of the pyrolysis system. As expected, an increase in temperature resulted in a decrease in biocarbon yield and an increase in gas yield for both birch and spruce feedstocks. The biocarbon yields from spruce are 32 wt.% and 26 wt.% for pyrolysis at 400 °C and 550 °C, respectively and are higher than the biocarbon yields observed for birch, 28 wt, % and 23 wt. % at corresponding temperatures. This might be an inherent effect of the different feedstocks as higher yields of charcoal are usually obtained from conifers than from deciduous trees (Wenzl, 2012). The different shapes of the wood chips, as can be seen in Fig. 1, may also have influenced biocarbon yields. Although sieved with the same sieves and in the exact same manner, the birch chips are thinner and shorter than the spruce chips (note, however, the treatment prior to fractionation was different for the two raw materials). The vapours generated within the spruce chips might have more time to form secondary biocarbon by decomposition before escaping the chips (Antal & Grønli, 2003). No clear trend in the changes of the mass yield of the liquid condensate phases was observed with the temperature changes.

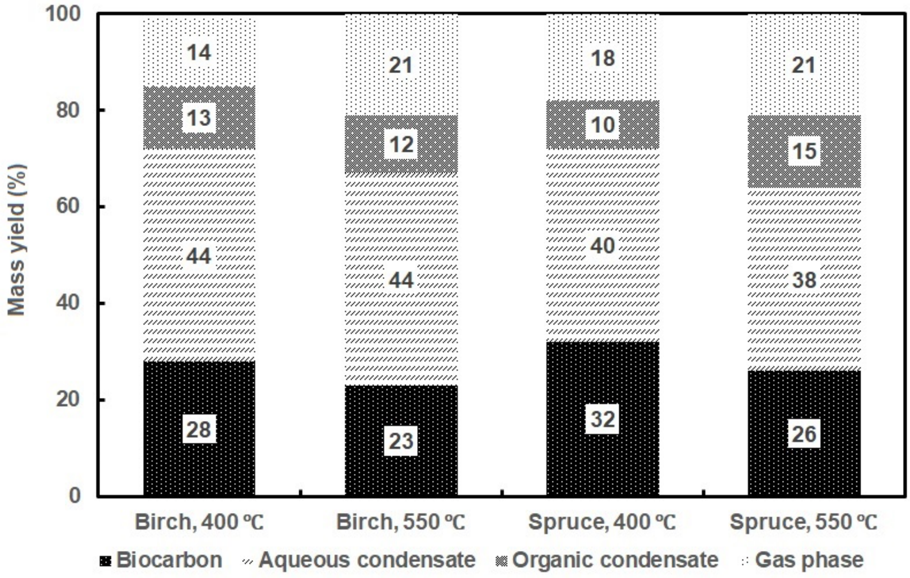

Fig. 3. Mass balances of pyrolysis of Birch and Spruce

The carbon distribution from the pyrolysis of birch and spruce at 400 °C and 550 °C is given in Figure 4. Similar trends for the biocarbon and gas carbon yields as for mass yields were observed. An increase in pyrolysis temperature results in a decrease in carbon yield in biocarbon and increases the carbon yield in the gas phase for both feedstocks. Similar decreases in the carbon content of the aqueous condensate with an increase in pyrolysis temperature is observed for both feedstocks. For birch, a decrease from 26 % to 23 % of the carbon is observed from the aqueous condensate with an increase in temperature, while a decrease from 16 % to 13 % was observed for the aqueous condensate of spruce. On the other hand, no consistent effect of the increase in pyrolysis temperature on the carbon yields in the organic condensates for the two feedstocks was observed. The organic condensate of spruce had a considerable increase in carbon yield from 15 % to 21 % with the increase in pyrolysis temperature, while a small decrease from 15 % to 14 % carbon yield was observed in the organic condensate for birch.

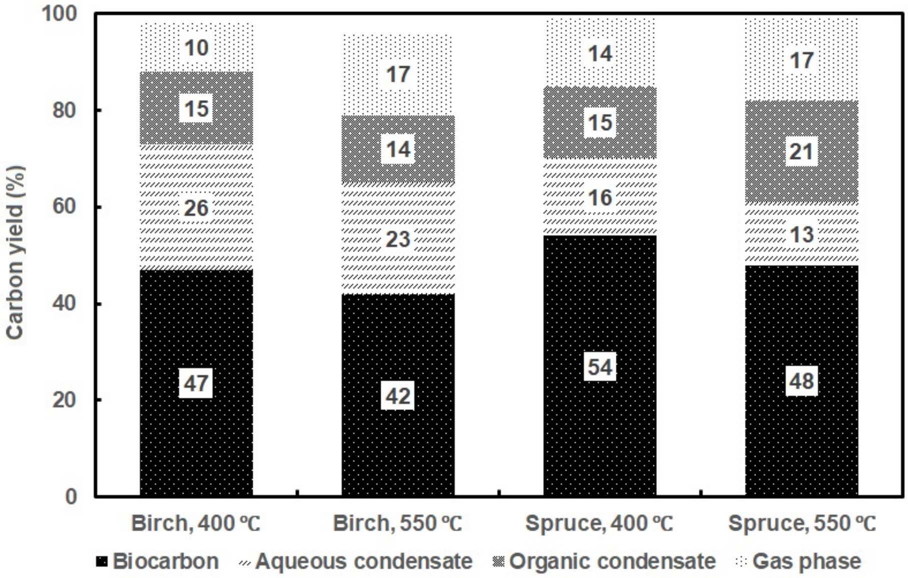

Fig. 4. Carbon yield of the pyrolysis products from Birch and Spruce.

Table 2 shows the molar gas composition of the 4 pyrolysis experiments. The main gas produced at 400 °C is CO_2, followed by CO, C1 to C3 hydrocarbons and H_2. Pyrolysis at 550 °C reduces the produced CO_2 fraction compared to pyrolysis at 400 °C for both birch and spruce. The other gas fractions are enriched with the increase in pyrolysis temperature, resulting in a higher energy content of the pyrolysis gas. The gas fraction can be combusted for process energy purposes, but the moisture content can limit the feasibility. Another possible application for the pyrolysis gas is to recycle the gas back into the process (Dhyani & Bhaskar, 2018).

Table 2. Pyrolysis gas composition.

Feedstock	CO_2 (mol %)	CO (mol %)	Methane (mol %)	Ethylene (mol %)	Ethane (mol %)	Propane+ propylene (mol %)	H_2 (mol %)
Birch. 400 °C	55.9	33.2	7.8	0.7	1.0	0.4	1.0
Birch. 550 °C	37.7	36.5	16.4	1.2	1.7	1.1	5.4
Spruce. 400 °C	50.3	37.2	9.1	0.7	1.3	0.5	0.9
Spruce. 550 °C	35.5	38.7	15.9	1.2	1.5	0.8	6.4

More carbon is retained in the aqueous condensates of birch than the aqueous condensates of spruce at the corresponding temperatures as can be seen from the carbon balance in Figure 4. This is most likely due to the higher content of hemicelluloses in birch (Dhyani & Bhaskar, 2018). Hemicelluloses are expected to promote the yield of the aqueous condensate since they degrade at lower temperatures compared to cellulose and lignin (Yang et al., 2007). The aqueous condensates of spruce are also opaquer than the aqueous phases from birch, as can be seen in Figure 5. This indicates higher water content and less concentrated carbon-containing substances in the aqueous phases of spruce. This is confirmed by water content measurements and elemental analysis presented in Table 3. The high amount of carbon retained in the aqueous phases, emphasizes the need for a suitable anaerobic digestion to complement the pyrolysis process to achieve high carbon utilization.

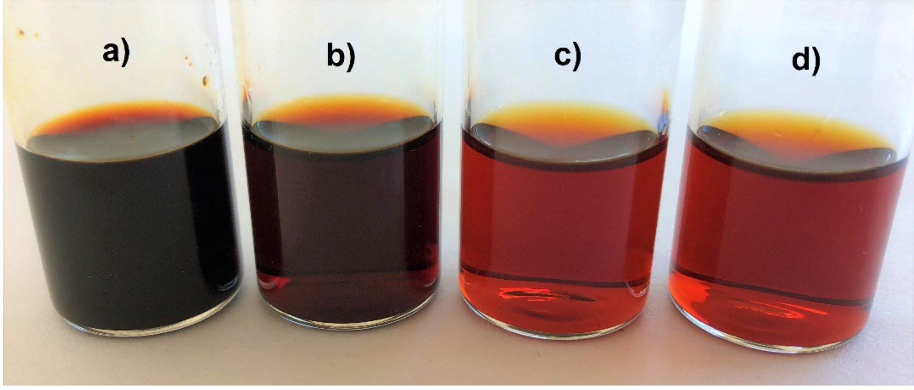

Fig. 5. Aqueous condensate samples for anaerobic digestion. a) Birch 400 °C. b) Birch 550 °C. c) Spruce 400 °C and d) Spruce 550 °C.

The effect of pyrolysis temperature on biomethane yield during anaerobic digestion

The aqueous condensate from the pyrolysis used as feedstock in AD gave methane yields of APL ranging from 112 NmL/gCOD to 207 NmL/gCOD depending on the type of biomass and the pyrolysis temperature (Table 3). APL of birch from pyrolysis at 400 °C and 550 °C had a biomethane yield of 44 % and 49 %, respectively, of theoretical achievable yield. A small increase in yield was observed with the increase in pyrolysis temperature for birch. Contrarily, a large decrease in methane yield from 59 % to 32 % was observed from the APL of spruce with the increase in pyrolysis temperature from 400 °C to 550 °C. A decrease in methane yield from APL with increasing pyrolysis temperature has also been observed by others (Erdogan et al., 2015; Hübner & Mumme, 2015).

The common trend for both birch and spruce is a decrease in methane yield with increased carbon content in the APL (Figure 6). Increase in pyrolysis temperature reduces the concentrations in APL of easily degradable ketones and acids while forming higher concentrations of recalcitrant or toxic carbon compounds such as hydroxyacetaldehyde, acetol, furans, N-heterocyclic compounds and phenols inhibiting bacteria and archaea, thus hampering the AD (Alvarez et al., 2014; Hierholtzer et al., 2013; Rezaei et al., 2014).

Table 3. Aqueous phase parameters and anaerobic digestion results

Feedstock	Water content (wt%)	Carbon content (wet basis) (wt%)	Carbon content (dry basis) (wt%)	COD (g/L)	Methane yield (gCOD/gCOD)/(NmL/gCOD)
Birch. 400 °C	46.5	28.0	52.2	703 ± 4	0.44 ± 0.01 / 152 ± 4
Birch. 550 °C	57.1	21.2	49.6	556 ± 3	0.49 ± 0.03 / 172 ± 8
Spruce. 400 °C	67.8	14.7	45.5	432.5 ± 0.7	0.59 ± 0.02 / 207 ± 10
Spruce. 550 °C	66.8	15.3	46.1	445 ± 1	0.32 ± 0.03/ 112 ± 11

The effect of carbon content on methane yield was large for Spruce (softwood). This is believed to be an effect of the high lignin content of Norway spruce (27.6-29.4 wt. % dry basis) (Părpăriţă et al., 2014). This leads to higher concentration of complex phenols with higher molecular weight with increased carbon content in spruce bio-oil (Stefanidis et al., 2014). On the other hand, the effect of carbon content on methane yield was low for Birch (hardwood) although the difference in carbon content between APLs pretreated at different temperatures was significant. This is explained by the lower lignin content (21 wt. % dry basis) (Părpăriţă et al., 2014) and by the more complex lignin structures of hardwood (syringil-guaiacyl lignin) compared to softwood (guaiacyl lignin) (Fahmi et al., 2008). The more complex lignin of hardwoods has a lower decomposition rate yielding less inhibitory APL (Torri et al., 2016).

The methane yield will likely be higher in a continuous AD process compared to batch tests as it allows microorganisms to adapt to the inhibitors present in the APL (Torri & Fabbri, 2014). Dilution and addition of additives such as biochar can also enhance AD and increase the methane yield (Torri & Fabbri, 2014).

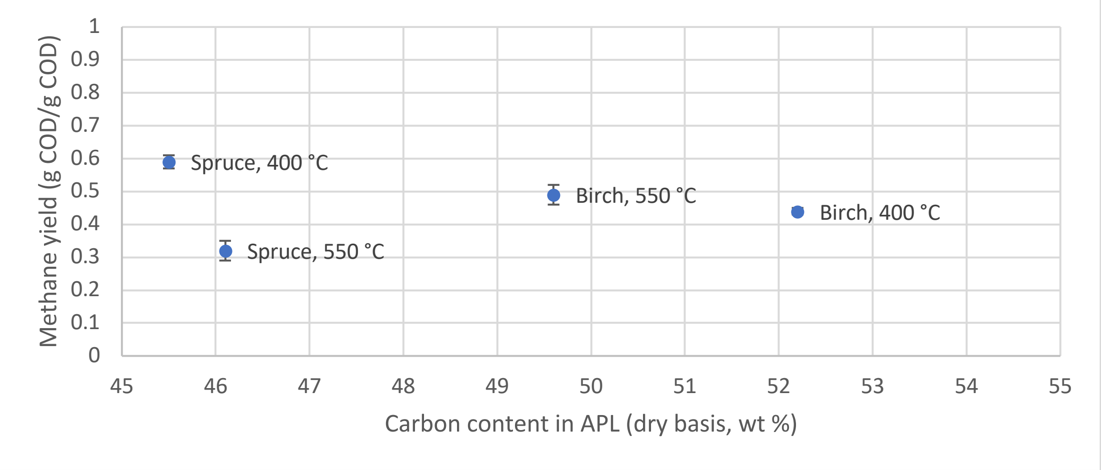

Fig. 6. Methane yield decreasing with increasing carbon content in APL.

Overall carbon balance for the current system

Figure 7 compares the combined utilised carbon yield for the four experiments with the chosen benchmark of 52.1 % carbon yield for conventional charcoal production, i.e.

the "hot tail" kiln. The utilised carbon yield contains all the carbon yields from the fractions obtained which can be used further, either as transportation fuel, energy carrier or reducing agent. It contains the carbon yields of biocarbon from pyrolysis, which can be used as a reducing agent; biomethane yield produced with AD of APL which can be used as transportation fuel; and organic condensate and combustible carbon-containing gasses generated during pyrolysis, which can be used as energy carriers. H_2 gas is also produced in some amount during pyrolysis, especially during the higher temperature pyrolysis. The H_2 adds to the energy content of the generated pyrolysis gas but is not included here.

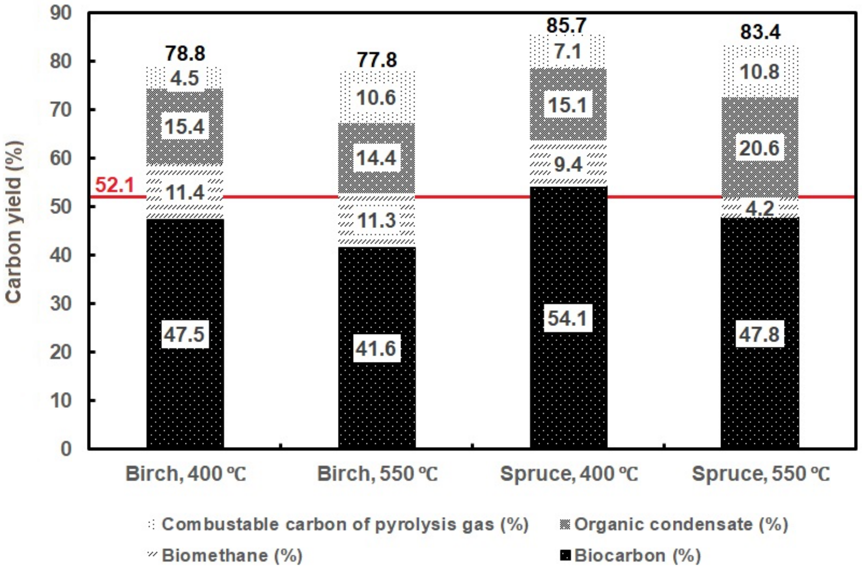

Fig. 7. Utilisation of carbon from the different fractions when combining pyrolysis and anaerobic digestion. The red line located at 52.1 % is the chosen benchmark for comparison.

All 4 systems yielded higher carbon utilisation compared to the benchmark of 52.1 % carbon utilisation of the "hot tail" kiln. Birch obtained combined carbon utilisation yields of 78.8 % and 77.8 % for pyrolysis temperatures of 400 °C and 550 °C, respectively. Spruce obtained slightly higher combined carbon utilisation yields of 83.4 % and 82.7 % for pyrolysis temperatures of 400 °C and 550 °C, respectively. The main factor for the lower total carbon yield of birch is the unconverted carbon in AD. Although obtaining reasonable biomethane yields for batch conversion, with 44 % and 49 % from APL from birch pyrolyzed at 400 °C and 550 °C, respectively, the high carbon retention in the APL of birch results in considerable carbon losses with the unconverted carbon with AD. More total utilised carbon was obtained with spruce than birch, even though the biomethane contribution was quite small for spruce, especially for spruce pyrolyzed at 550 °C due to low carbon retention in the aqueous condensate of spruce pyrolysis.

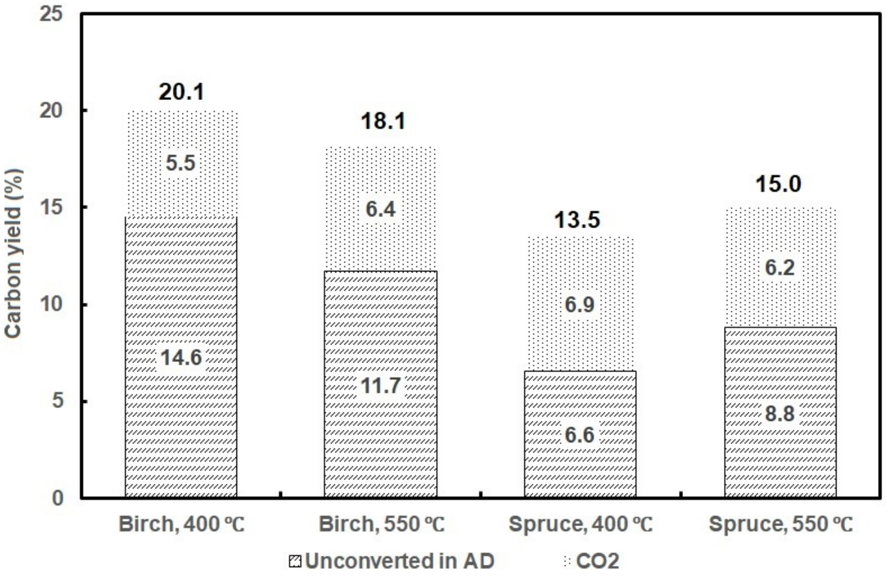

Fig. 8. Carbon losses of the combined system of intermediate pyrolysis and anaerobic digestion.

The carbon losses identified for this system are given in Figure 8. The two main carbon losses are CO_2 generation during pyrolysis and unconverted carbon during AD. The largest carbon loss in all cases is unconverted carbon of APL, except for spruce pyrolyzed at 400 °C which had the highest biomethane yield of 59 %. The other experiments had carbon recovery to biomethane of less than 50 %. Developing efficient AD processes with adapted microorganisms should be carried out to further increase the overall carbon utilisation. The carbon loss to CO_2 is similar for birch and spruce at 400 ℃ and 550 ℃.

The biochar from spruce pyrolyzed at 400 °C attained a carbon yield of 54.1 % exceeding the chosen benchmark of 52.1 %. The high carbon yield of biochar from spruce might be due to the higher expected yield from conifers as discussed before. Nevertheless, substantial amounts of biocarbon was still produced in the other three experiments. This shows that with a small sacrifice in biocarbon yield, biocarbon can be produced with much shorter process times with intermediate pyrolysis compared to conventional charcoal production. In addition, intermediate pyrolysis allows for collecting and utilising viable side streams to obtain increased carbon utilisation by generating energy carriers, organic condensate and pyrolysis gas, and producing transportation fuel, biomethane, by AD of APL.

CONCLUSIONS

1. Intermediate pyrolysis has enhanced carbon utilisation potential compared to conventional charcoal production methods if all fractions produced during pyrolysis are utilised.
2. Spruce has a higher inherent potential for high biochar yields than birch, while birch has higher carbon retention in the aqueous condensate (APL), thus birch is more dependent on good carbon recovery in terms of producing biomethane by anaerobic digestion for high total carbon utilization.

3. As shown in batch experiments, anaerobic digestion may be used to recover reasonable amounts of carbon as biomethane from the aqueous condensate from intermediate pyrolysis of wood. This shows the potential of high carbon recovery from APL with well adapted microorganisms, continuous systems and enhancing additives.
4. A decrease in biomethane yield was observed for spruce with increased pyrolysis temperature, something which may be explained by increased formation of inhibitors from softwood lignin. A decrease in biomethane yield was not observed with increased pyrolysis temperature for birch.
5. A decrease in methane yield with increased carbon content in the APL was observed for both birch and spruce. The effect is largest for spruce and is associated with the less complex softwood lignin leading to higher concentrations of recalcitrant and toxic compounds in the intermediate pyrolysis APL.

ACKNOWLEDGMENTS

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Article 4

Methane Production in Cascade Processing of Woody Biomass

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Methane Production in Cascade Processing of Woody Biomass

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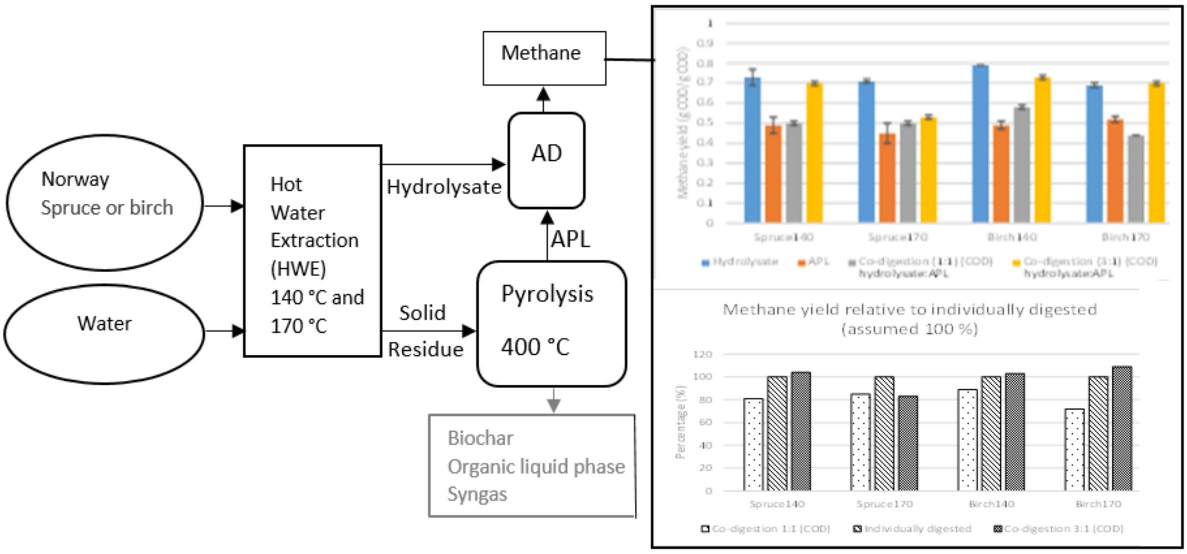
Highlights

- Hot water extraction pretreatment severity influences methane yield from the extract
- No effect of HWE pretreatment severity before pyrolysis on methane yield of APL
- APL as an additive to hydrolysate in AD gives the same methane yield as the sum of individual digestion but improves the digestion rate during co-digestion

Keywords

Lignocellulosic woody biomass, Hot water extraction (HWE), Pyrolysis, Hydrolysate, Aqueous pyrolysis liquid (APL), Anaerobic digestion (AD)

Graphical Abstract



Abstract

Lignocellulosic woody biomass can be used for bio-based fuel and material production, such as bio-methane and biochar, to mitigate negative effects of using fossil-based sources. In a cascade processing, Norway spruce (softwood) and birch (hardwood) were hot water extracted (HWE) at two conditions (170 °C for 90 minutes (severity 4.02) and 140 °C for 300 minutes (severity 3.65)) before intermediate pyrolysis at 400 °C producing organic rich hydrolysate and aqueous pyrolysis liquid (APL) respectively as waste streams while producing biochar as the main product. Batch anaerobic digestion (AD) of hydrolysate and APL, both individually and co-digested, was performed to study their methane potential. Hydrolysate showed good biodegradability (around 70 %) compared to APL (just around 50 %) suggesting higher suitability of sugar rich hydrolysate to AD than APL which contains several toxic and inhibitory compounds. Hydrolysate prepared at 140 °C compared to 170 °C had better methane yield for birch while the pretreatment temperature had no significant effect on Norway spruce. Effect of HWE temperature on methane yield of APL was low, while HWE temperature of 140 °C resulted in longer digestion time than 170 °C for both Norway spruce and birch. Co-digestion of APL with hydrolysate improved the overall methane yield at a chemical oxygen demand (COD) based feed ratio of 3:1 (hydrolysate:APL) compared the ratio of 1:1, while the improvement was not significant compared to individual digestion. This suggests the use of APL as an additive substrate in moderate ratios to hydrolysate can be advantageous considering the toxicity of APL and high biodegradability of hydrolysate and typical ratios generated under such process scheme.

1 Introduction

There is a demand for clean and renewable sources for energy and products due to fossil fuel's uncertainty, unsustainability and environmental and human health concerns (Wang et al., 2018). Metallurgic industries are one of the consumers of fossil coal as a reducing agent and energy source and replacing it with renewable bio-based coal could overcome environmental problems posed by fossil-based coal. By introducing new bio-products, the biomass processing should be optimized with enhanced energy recovery to make it economical viable. Biomass cascade processing by hot water extraction, pyrolysis and anaerobic digestion can be a solution for renewable bio-based coal production combined with bio-methane production.

Pyrolysis is a modern technology fundamentally similar to traditional high CO$_2$ impact charcoal production (Bailis, 2009) in which thermal degradation of biomass takes place in oxygen free environment. It has been regarded as a promising route for biomass utilization (Maschio et al., 1992). A shift from the initial focus on bio-oil production to co-production of bio-oil and biochar in the recent years comes with the recognition of many applications and potential environmental benefits of biochar (Bridgwater, 2012; Cha et al., 2016). A cascade processing targeted to improve the biochar as the main product while maintaining a focus on the total energy recovery is relatively new.

The bio-oil from intermediate pyrolysis usually separates into two phases, an organic condensate phase and an aqueous pyrolysis liquid (APL). The organic phase can be used as renewable fuel after upgrading (Oasmaa et al., 2015; Pollard et al., 2012); and also for other energy purposes, chemical production, bio-based pesticides and in manure acidification (Hagner et al., 2018; Keskinen et al., 2017; Rasi et al., 2019). The aqueous phase APL can contain a significant fraction of carbon such as sugar derivatives and other water soluble organics, but is too dilute for recovery of its organic content cost-effectively (Zhou et al., 2019). It also contains compounds which requires effective treatment for environmental concerns (Evans and Milne, 1987; Huber et al., 2006; Kan et al., 2017; Liaw et al., 2015; Rezaei et al., 2014). The use of APL has also been limited due to its acidity, chemical and thermal instability (Kan et al., 2017; Zhou et al., 2019).

Anaerobic digestion (AD) is recently examined as an option to handle APL. AD is a biological process in which bacteria and archaea break down organic substrates into biogas (~50-75 % CH$_4$ and 50-25 % CO$_2$) in the absence of free oxygen. Mixed communities of organisms makes it a robust technology with capabilities of AD microorganisms to adapt to a wide range of chemical substances (Torri and Fabbri, 2014) and can handle various complex, recalcitrant, and inhibiting compounds (Benjamin et al., 1984; Vasco-Correa et al., 2018). APL, despite being a complex substrate with around 300 compounds where a few compounds are toxic to microorganisms even in small concentrations, can potentially be a good feedstock for biological valorisation (Torri et al., 2020).

Cascade processing is considered for enhanced carbon efficiency and production of higher value products. To achieve this various pretreatments can be applied on the biomass feedstock before pyrolysis based on the composition of the lignocellulosic material. Hardwood and softwood are composed of cellulose (30-50 %), hemicellulose (20-30 %) and lignin (20-40 %) and generally a small fraction of inorganic mineral matter (McKendry, 2002). Lignin is the desired fraction for solid biochar production while cellulose and hemicellulose contribute in bio-oil production (Akhtar and Saidina Amin, 2012). Hot water extraction (HWE) is one of the simplest and most cost-effective hydrothermal pretreatment, carried out in the temperature range of 120 – 230 °C and at various pressure conditions at which water is kept in subcritical conditions (Nitsos et al., 2013). It removes hemicellulose significantly along with a small part of lignin resulting in a solid residue with a higher content of lignin and cellulose. Removing hemicellulose before pyrolysis reduces the concentration of acids and furans in bio-oil, which are inhibitory to microorganisms in higher concentration (Alvarez-Chavez et al., 2019). It also substantially removes inorganic metal from the biomass, which could have negative effect in pyrolysis chemistry, and produces quality bio-oil with higher heating value (HHV) because of increased concentration of anhydrosugars and decreased concentration of water present in bio-oil (Kumar et al., 2020).

During the HWE process, a side-stream of aqueous hemicellulose (or hydrolysate) is produced which mainly consists of pentose and hexose sugars like xylose, glucose, mannose, arabinose and galactose (Mosier et al., 2005b). Acetic acid is the most abundant volatile fatty acid (VFA) produced in the hydrolysate which acts as a catalyst to degrade the polymers for increased sugar yields (Hu and Ragauskas, 2012). It is important to utilize this organic rich liquid stream to enhance energy recovery and avoid environmental pollution. As the hydrolysate is pentose rich, bioethanol production is not considered suitable due to the requirement of genetically modified microorganisms thriving on pentose combined with a high sensitivity of the microorganisms to inhibitors (Kaparaju et al., 2009; Torry-Smith et al., 2003). Although the hydrolysate has been previously considered for the production of health food additive, acetic acid, ethanol, butanol, lactic acid, polyhydroxyalkanoates (PHA) and the production of hydrogen through dark fermentation the results were not encouraging (Kadar et al., 2004; Kongjan et al., 2010; Liu, 2015; Walton, 2009). Hydrolysate is a suitable feed for AD due to presence of higher sugar concentration and lower level of inhibitors, as HWE operates at low temperature with large water volume. However, several inhibitors such as furfural, 5-hydroxy methyl furfural (HMF), soluble lignin, and its derivatives are produced during HWE, especially at higher temperatures, which makes it more challenging (Monlau et al., 2014; Mosier et al., 2005a).

AD can recover carbon from sugar rich hemicellulose hydrolysate and organic rich APL produced by HWE and pyrolysis, respectively, despite the inhibitors present in them. Since AD produces methane, a clean drop-in fuel, it is considered to potentially be an effective waste treatment technology of these waste streams (Appels et al., 2008; Khalid et al., 2011). Co-digestion is potentially an effective strategy during AD to increase methane yield by overcoming various shortcomings of mono-digestion resulting in process stabilization, dilution of inhibitory substance and synergetic effect of microorganisms (Hagos et al., 2017; Yu et al., 2020). Although co-digestion of hydrolysate and APL of agricultural residues and energy crops have been studied, AD of hydrolysate and APL from woody biomass is still very limited. The suggested cascade processing has only been performed on waste substrates like sewage sludge (Li et al., 2018) and softwood bark (from Norway spruce and Scots pine) (Rasi et al., 2019) while woody biomass, especially Norway spruce and birch, has not yet been considered for this concept. Moreover, cascade processing targeted to produce an improved biochar as the main product is relatively new.

The goal of this research is to enhance energy recovery of aqueous side streams by finding potential synergies in co-digestion of hydrolysate and APL. Two HWE severity factors were chosen to extract hydrolysate from Norway spruce and birch. The HWE pretreated chips were subsequently pyrolyzed to corresponding APL samples. Hydrolysate from the HWE pretreatment and APL from the pyrolysis are then both individually digested and co-digested in AD and compared for methane production.

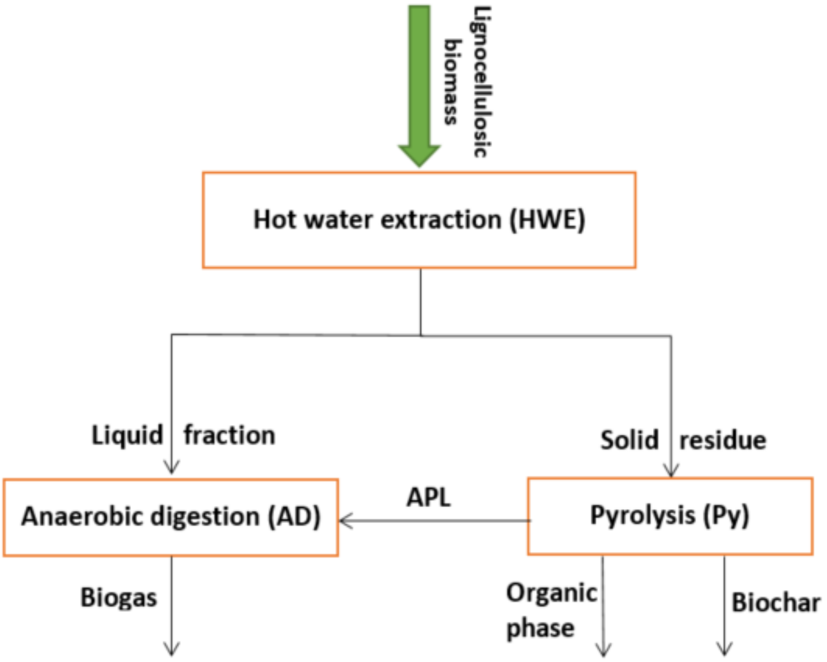


Figure 1. Flow chart showing processing of Norway spruce and birch, producing biogas, bio-oil (APL and organic phase) and biochar, illustrating the process examined in this study.

2 Material and methods

2.1 Raw materials

Norway spruce and birch woodchips were used in this experiment. The Norway spruce chips had a dry matter content of 44.5 % when it were received from a Norwegian forestry company, and the sample was air dried to 93.9 %. Birch chips were received dry from a Norwegian sludge refining company, with a dry matter content of 93.4 %. Both samples were hammer milled with 1000 RPM through a 19 mm hole screen and fractionated to a size between 13 mm and 5 mm.

2.2 Hot water extraction

The wood chips were mixed with distilled water in a 1:5 weight ratio and hot water extracted (HWE) in a Mini-Mill Laboratory Digester (MMLD) from MK Systems Inc. The mixture were loaded into the MMLD before preheated to 110 ℃ and kept at this temperature for 20 minutes. The temperature was then increased to the target temperature of 140 ℃ or 170 ℃ over the course of 20 or 30 minutes, respectively, while the final temperature was kept for 300 minutes or 90 minutes, respectively. After the HWE, the hydrolysate was first collected before the wood chips were rinsed thoroughly with water and air-dried to approximately 95 % dry matter.

Measurements of the carbohydrate composition and concentrations of furfural and HMF in the hydrolysates were carried out as described in Ghimire et al. (2020). In order to evaluate the combined effects of pretreatment time and temperature for each treatment, a severity factor (Equation 1) is calculated (Overend and Chornet, 1987) (Table 1). It is particularly useful while comparing pretreatment effects.

$$\text{Severity factor} = \log(R_0) = \log(t \times \exp(\frac{T-100}{14.75})) \tag{1}$$

Where, T (°C) is the pretreatment temperature and t (min) is the reaction time.

Table 1. Severity factor for the hydrolysates from HWE.

Feedstock	Hydrothermal pretreatment conditions		Severity factor log(R_0)
	Temperature (°C)	Time (min)	
Norway spruce170 and Birch170	170	90	4.02
Norway spruce140 and Birch140	140	300	3.65

2.3 Pyrolysis

A Pyrolysis Development Unit (PDU) (Figure 2) at RISE PFI AS was used to pyrolyze the air dried hot water extracted wood chips. A detailed description regarding the pyrolysis rig and process can be found in van der Wijst et al. (2021).

The vapors from the pyrolysis were condensed in water condensers before they naturally phase separated into an aqueous pyrolysis phase and an organic phase. The top phase APL was decanted off from the condensate after stored in the condensate bottles overnight.

Water and carbon content in APL were measured with Karl-Fischer titration on a Mettler Toledo V20 Volumetric KF Titrator and a Thermo Scientific Flash 2000 CHNS/O Analyzer respectively.

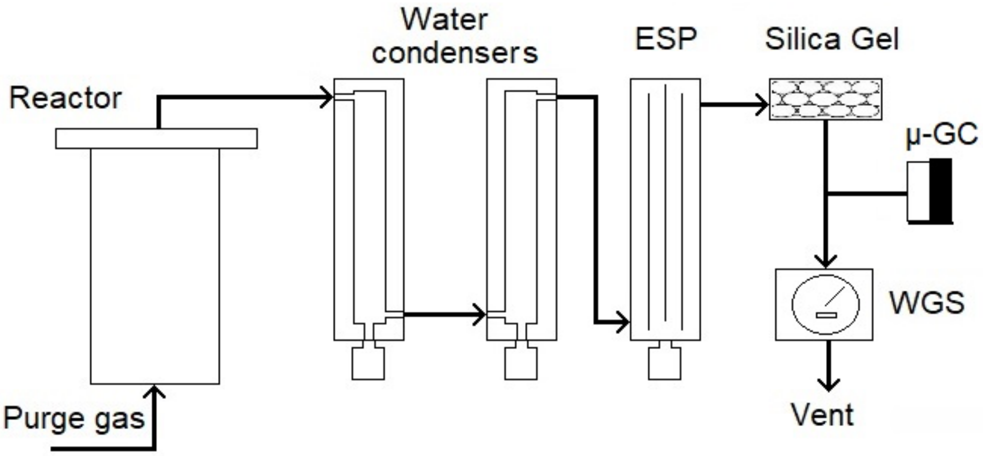


Figure 2. Sketch of the pyrolysis rig at RISE PFI (ESP- Electrostatic precipitator; WGS-Water gas shift)

2.4 Anaerobic digestion

The hydrolysates from HWE and the condensates APL from pyrolysis were tested for bio-methane potential (BMP) during batch anaerobic digestion in an Automatic Methane Potential Test System II (AMPTS II, Bioprocess Control® Sweden AB), a standardized laboratory set-up designed to determine BMP of any organic material (Badshah et al., 2012).

The feed substrates (hydrolysates and APL) were mixed with a macro nutrient solution and an inoculum. The macro nutrient solution was added in the AD feed substrates to maintain a minimum COD:N:P ratio of 350:5:1 (Baeta et al., 2013). The macro nutrient solution was composed of NH_4Cl (44.48 g/L), $(NH_4)H_2PO_4$ (5.3 g/L), $(NH_4)_2HPO_4$ (1.78 g/L), $MgCl_2 \cdot 6H_2O$ (21.4 g/L), $CaCl_2 \cdot 2H_2O$ (7.56 g/L) and $NaHCO_3$ (100 g/L). The inoculum used was a mesophilic granular sludge originally obtained from a mesophilic industrial internal recirculation reactor treating paper mill effluent. The inoculum total solid and volatile solid concentrations were 181 and 119.4 g/L. The inoculum was degassed at 30°C for at least 5 days before used for the experiment to reduce the gas production from the inoculum. The amount of feed added to the inoculum, the organic load (OL), was 10 g COD/L for the hydrolysates and 5 g COD/L for the APL when digested individually and 7.5 g COD/L when co-digested together at two different load ratios for hydrolysate:APL (Table 2, Table 3, Table 4, Table 5). Each sample was run in triplicates at 35 °C. The system was purged with pure nitrogen gas for 3 minutes to ensure desired anaerobic condition. The detailed process is described elsewhere (Ghimire et al., 2020).

Blank sample, only containing inoculum, was tested in triplicates along with the investigated samples. Gas produced from blank sample was deducted from the gas produced from hydrolysates to offset the gas produced by endogenous respiration of microorganisms in inoculum. The results thus represent only the gas produced from the tested samples.

Table 2. AMPTS II experimental design for AD of hydrolysates from HWE.

Feedstock	Inoculum (mL)	Hydrolysate (mL)	Organic Load (g COD/L)
Spruce140	200	70	10
Spruce170	200	44	10
Birch140	200	39.5	10
Birch170	200	28	10
Control (Blank)	200	70 (distilled water)	Not Applicable

Table 3. AMPTS II experimental design for AD of APL after HWE and pyrolysis.

Feedstock	Inoculum (mL)	APL (mL)	Organic Load (g COD/L)
APLSpruce140	200	1.2	5
APLSpruce170	200	1.45	5
APLBirch140	200	1.45	5
APLBirch170	200	1.6	5
Control (Blank)	200	1.6 (distilled water)	Not Applicable

Table 4. AMPTS II experimental design for co-digestion AD of hydrolysate (after HWE) and APL (after HWE and pyrolysis) at equal COD loading of 1:1 (Hydrolysate:APL).

Feedstock	Inoculum (mL)	Hydrolysate (mL)	APL (mL)	Organic Load (g COD/L)
CoAPLSpruce140 (1:1)	200	26.3	1.07	7.5
CoAPLSpruce170 (1:1)	200	16.5	1.19	7.5
CoAPLBirch140 (1:1)	200	14.8	0.9	7.5
CoAPLBirch170 (1:1)	200	10.46	1.08	7.5
Control (Blank)	200	25 (distilled water)		Not Applicable

Table 5. AMPTS II experimental design for co-digestion AD of hydrolysate (after HWE) and APL (after HWE and pyrolysis) at COD loading of 3:1 (Hydrolysate:APL).

Feedstock	Inoculum (mL)	Hydrolysate (mL)	APL (mL)	Organic Load (g COD/L)
CoAPLSpruce140 (3:1)	200	39.4	0.54	7.5
CoAPLSpruce170 (3:1)	200	24.8	0.59	7.5
CoAPLBirch140 (3:1)	200	22.2	0.45	7.5
CoAPLBirch170 (3:1)	200	15.7	0.55	7.5
Control (Blank)	200	25 (distilled water)		Not Applicable

CODs was measured according to US standard 5220D using commercial kits (WTW^{TM}) (Federation, 1995) after filtering samples through a 0.45 µm pore size glass filter. pH was measured using WTW inolab pH7110. VFA concentrations were carried out as described in Ghimire et al. (2020).

2.5 Statistical analyses

Statistical analyses were done using the one-way analysis of variance (ANOVA) using MS-Excel 2016. Statistical significance was denoted at $P < 0.05$.

3 Results and discussions

3.1 Hot water extraction (HWE) - Effect of feedstock and temperature on hydrolysate

The content in the hydrolysates are influenced by the type of wood; birch (hardwood) or spruce (softwood), as well as the extraction temperature; 140 °C or 170 °C. This is first examined based on the measured chemical oxygen demand (COD), sugar, VFA and inhibitor concentrations.

The soluble COD (CODs) and total COD (CODt) concentrations increased with pretreatment temperature for both wood hydrolysates (Figure 3a), with concentration of Norway spruce treated at 140 °C of 27.6 g CODs/L and 28.5 g CODt/L compared to 42.4 g CODs/L and 45.4 g CODt/L of Norway spruce treated at 170 °C. Similarly, for birch treated at 140 °C was 42 g CODs/L and 50.6 g CODt/L compared to 65.6 g CODs/L and 71.7 g CODt/L respectively of birch treated at 170 °C. This suggests that soluble organics dominates in hydrolysates with rather similar CODs and CODt values where the solubilization of organic substances increased with increase in temperature and were higher for hardwood compared to softwood. The effect from wood type is also observed by others and attributed to higher amount of acetyl groups linked to the hemicelluloses in hardwood and also a good delignification efficiency for hardwood (Sixta, 2006).

The sugars found in the hydrolysates were the hemicellulosic sugars arabinose, galactose, glucose, xylose and mannose. The total sugar concentration was higher in hardwood hydrolysate in both pretreatment temperatures (Figure 3b) with a total sugar concentration of 20.8 g/L and 26.5 g/L in birch hydrolysate compared to 11.9 g/L and 19.9 g/L in Norway spruce hydrolysate at pretreatment temperature of 140 °C and 170 °C respectively. This can be attributed to a lower resistance of hardwood hemicelluloses to hydrothermal treatment compared to softwood (Ramos, 2003).

The composition of the sugars in the hydrolysate also depended on the types of the biomass. For hardwood xylose was the main sugar with 80 % of the total sugar content (with minor contribution of arabinose, galactose, glucose and mannose) in the birch hydrolysates treated at both 140 °C and 170 °C. For softwood it was mannose (with low concentration of arabinose, galactose, glucose and xylose) which accounted for 40 % and 55 % in Norway spruce treated at 140 °C and 170 °C respectively, similar to observations by others (Leppänen et al., 2010; Testova et al., 2011). The concentration of glucose is found to be low in both types of wood and the value increases with the increase of pretreatment temperature. Usually, glucose is present in the hydrolysate due to hydrolysis of cellulose and as the HWE is not severe enough to cause dissolution of cellulose, it is expected to have originated from extractives or hemicelluloses (Nitsos et al., 2013; Rättö et al., 1993).

Higher sugar content in the hydrolysate is however not necessarily the best condition for methane production despite claims by different researchers (Baeta et al., 2016; Costa et al., 2014). A higher sugar content comes from increased dissolution of hemicellulosic sugars due to increasing pretreatment severity (temperature and pressure) that also increasingly forms degradation products like furfural and HMF inhibiting AD (Monlau et al., 2014). Furfural and HMF were formed in the hydrolysates as degradation products from pentose (xylose and arabinose) and hexose (mannose, glucose and galactose) sugars respectively (Cai et al., 2014). The concentrations of furfural were 1.6 g/L and 5.4 g/L compared to only 0.8 g/L and 0.1 g/L of HMF for Norway spruce and birch respectively when pretreated at high temperature. Higher furfural concentration in birch hydrolysate is attributed to higher xylose sugar (pentose) concentration in hardwood (Figure 3). Slightly higher HMF concentration in Norway spruce pretreated at 170 °C is due to higher mannose (hexose) concentration in Norway spruce (softwood).

Weak acids such as acetic acid and formic acid (not measured here) are generally produced in hydrolysate during hydrothermal pretreatment of lignocellulosic biomass (Mohapatra et al., 2017; Yousefifar et al., 2017). The acetic acid concentration was here the dominant volatile fatty acid, higher in hydrolysate prepared at high pretreatment temperature in both types of biomass (Figure 3d) with 166 % higher in hardwood and 107 % higher in softwood. Acetic acid is important as it is produced by degradation of acetyl residues from xylan hemicellulose (Palonen et al., 2004; Rabemanolontsoa and

Saka, 2016) creating an oxidative acidic environment which catalyzes the hemicellulose solubilization (Mohapatra et al., 2017; Yousefifar et al., 2017) making the acetic concentration higher in hardwood as the acetyl content is higher in hardwood (Nitsos et al., 2013). Acetic acid is also a key substrate for methane production as long as its concentration in the AD process is not above threshold value of inhibition (2400 mg/L) (Wang et al., 2009).

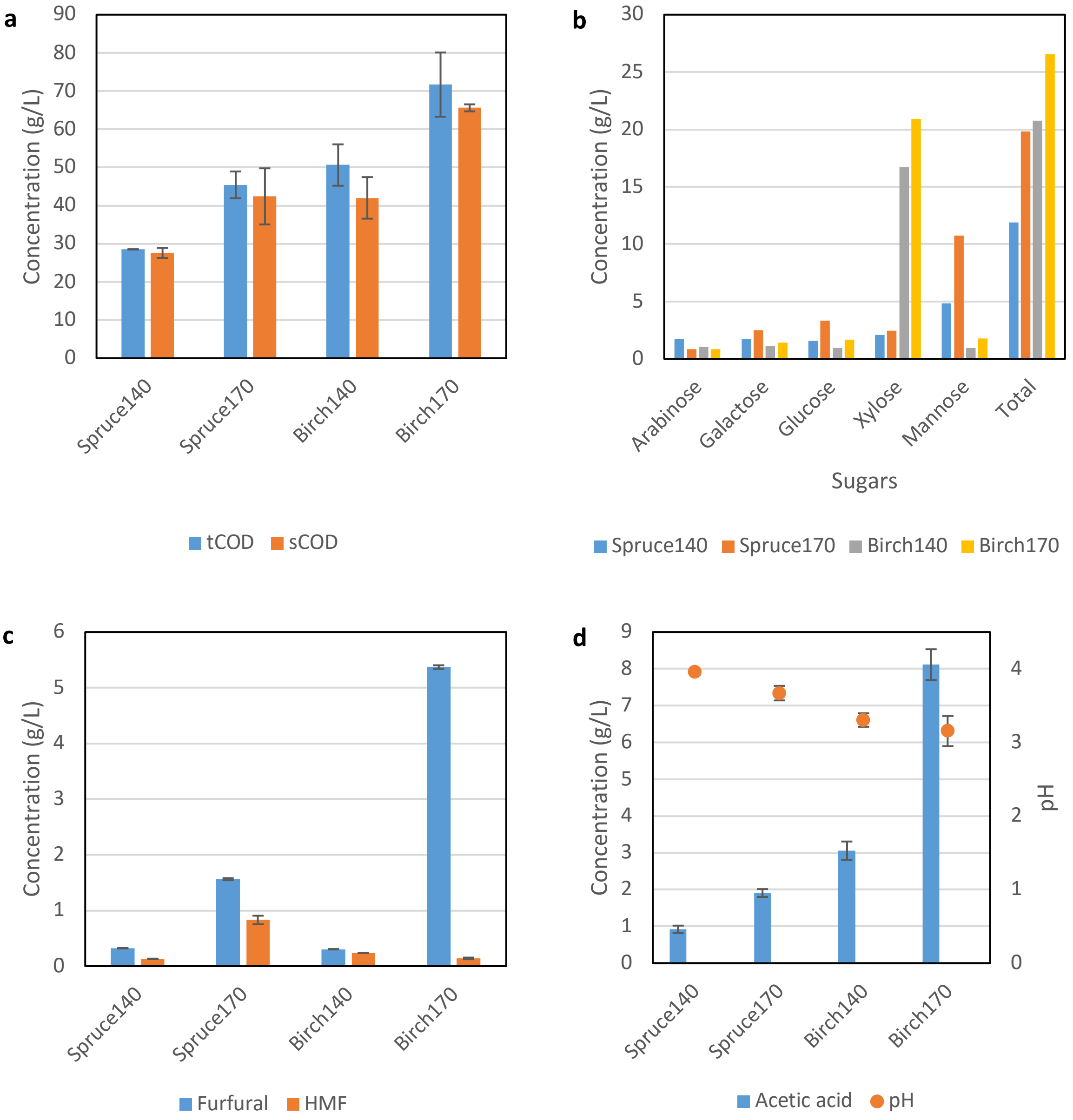


Figure 3. The effect of feedstock and hot water extraction temperature on composition of hydrolysate: (a) CODt and CODs (b) sugar (c) furfural and HMF (d) acetic acid and pH.

3.2 Pyrolysis - Effect of feedstock and HWE temperature on APL

The characteristics of the obtained APLs from HWE pretreated wood chips show that an increased temperature of the HWE pretreatment reduces the organic material present in APL (Table 6). The carbon content in the APL is reduced from 25.4 wt. % to 19.2 wt. % for Norway spruce, and from 33.0 wt. % to 26.9 wt. % for birch with an increase from 140 °C to 170 °C in HWE pretreatment temperature. The same trend is observed for the COD values of the APLs which decreases from 699 g/L to 630 g/L for Norway spruce and from 836 g/L to 695 g/L for birch. The reason is that an increase in the HWE temperature increases the extraction during the HWE pretreatment of the wood chips and reduces the easy degradable compounds of the wood chips, i.e. the hemicelluloses and some lignin, which products would otherwise end up in the APL.

Table 6. Content of aqueous pyrolysis liquid (APL).

Feedstock	Water content (wt%)	Carbon content (wet basis) (wt %)	Hydrogen content (wet basis) (wt %)	Nitrogen content (wet basis) (wt %)	Oxygen content, by difference (wet basis) (wt %)	COD (g/L)
APLSpruce140	55.3 ± 0.4	25.4	5.8	0	68.8	699
APLSpruce170	62.1 ± 0.2	19.2	6.3	0	74.5	630
APLBirch140	44.6 ± 0.5	33.0	6.6	0	60.4	836
APLBirch170	52.2 ± 0.3	26.9	7.0	0	66.1	695

This effect is highlighted by the VFA analysis. The high amount of hemicelluloses remaining in the wood chips after the low temperature HWE pretreatment leads to a higher potential of acid formation during pyrolysis. A clear increase in both total acid and acetic acid concentrations are measured in the ALP with the decrease in HWE pretreatment temperature (Table 7). Similar effects are also expected for lignin derived phenolic compounds. Higher concentration of inhibiting phenolic compounds are expected in the APL from the low temperature HWE pretreatment (Kan et al., 2016).

Table 7. Volatile fatty acid (VFA) concentration of APL.

Feedstock	Volatile fatty acids (VFAs) (g/L)			
	Acetic acid	Propionic acid	Isobutyric acid	Total
APLSpruce140	45.1 ± 1.9	3.1 ± 0.2	4.2 ± 0.2	77 ± 6
APLSpruce170	35.0 ± 0.6	3.4 ± 0.2	3.6 ± 0.1	59 ± 1
APLBirch140	96.2 ± 2.6	4.7 ± 1.0	3.8 ± 1.8	128 ± 6
APLBirch170	55.3 ± 0.9	5.6 ± 0.3	4.4 ± 1.5	84 ± 3

3.3 Anaerobic digestion

3.3.1 AD of hot water extract hydrolysate

The methane production from HWE hydrolysates of Norway spruce and birch produced at 140 °C and 170 °C was monitored for 19 days.

Two distinct peaks was observed in the methane production rate (Figure 4a and 4b), as also observed by others (Buitrón et al., 2019), and is due to different digestible fractions present in the hydrolysates. The first peak, contributing to 0.33 – 0.36 g COD/g COD methane yield, is attributed mainly to easily degradable components such as hemicellulosic sugars and acetic acid, representing around 67 % of the sugar and acetic content in the hydrolysates. A second peak after a short lag phase is observed in all the samples, which can be attributed to the methane production from breakdown of toxic, inhibiting and recalcitrant compounds produced during HWE pretreatment. Increasing the severity (Equation 1) during pretreatment leads to a higher formation of complex recalcitrant compounds and more extraction of soluble lignin into hydrolysates (Liu, 2015; López González et al., 2014). This complex recalcitrant compounds and soluble lignin can remain undigested, be slowly degraded or act as inhibitors during AD (Koyama et al., 2017; López González et al., 2014). This is presumably the reason for the observed longer lag phase between the two methane production peaks and also higher undigested CODs values (9% and 18% higher for Norway spruce and birch respectively) in the digestate at the end of the AD experiment for hydrolysates produced at high pretreatment temperature.

The HWE hydrolysates from increasing pretreatment temperature resulted in decreasing methane yield for birch (not significant decrease for Norway spruce). The methane yield of Norway spruce hydrolysate had values of 0.73 ± 0.04 g COD/g COD (253.7 NmL/g COD) and 0.71 ± 0.01 g COD/g COD (248.7 NmL/g COD) for pretreatment temperature of 140 °C and 170 °C respectively. Slightly higher methane yield of 0.79 ± 0.00 g COD/g COD (276.8 NmL/g COD) was observed in case of birch hydrolysate pretreated at 140 °C compared to only 0.69 ± 0.01 g COD/g COD (241.2 NmL/g COD) at pretreatment temperature of 170 °C. The methane yields observed here are in the range of values from hydrolysates of different types of agricultural residues ranging from 200 ml CH_4/g COD to 310 ml CH_4/g COD depending on different pretreatment severity (Costa et al., 2014; Eskicioglu et al., 2017; Fernandez-Cegri et al., 2012). Hemicellulose extraction is better in hardwood (birch) compared to softwood (Norway spruce) due to its higher content of hemicellulose, type of hemicellulose (acetylated xylans), lower lignin content and the more complex nature of the lignin component (Pan et al., 2005; Vivekanand et al., 2013) which resulted in a higher methane yield for birch at low pretreatment temperature. However, higher hemicellulose concentration in hardwood also leads to a higher concentration of degradation product at high pretreatment temperature with products hampering the methane yield as observed in birch pretreated at 170 °C. As synergistic inhibition occurs when different inhibitors are present together reducing considerably the inhibition threshold value, presence of HMF and furfural along with soluble lignin might have affected the AD process although the concentration of individual inhibitor is below the threshold value (0.8 mg/L for HMF and 2 g/L for furfural) (Ghasimi et al., 2016; Mussatto and Roberto, 2004).

Absence of a lag phase during the start of the methane production suggests that the microorganisms in the inoculum consumed the easily digestible components in the HWE hydrolysate while the microorganism took some adaptation time before digesting the remaining xylooligomers (XOS), toxic and inhibitory compounds broken down during the second peak (Benjamin et al., 1984). The microorganisms has difficulty in hydrolyzing xylooligomers (XOS), which represents around 30 % of the total COD of hydrolysates (Adarme et al., 2019; Baeta et al., 2016), into monosaccharides as microorganisms of AD consortium are not adapted to XOS and have to synthesize hydrolytic enzymes to breakdown these compounds. The methane yield is expected to be improved in a continuous system with microbial adaptation to XOS, as well as to the toxic and inhibitory compounds present in the feed.

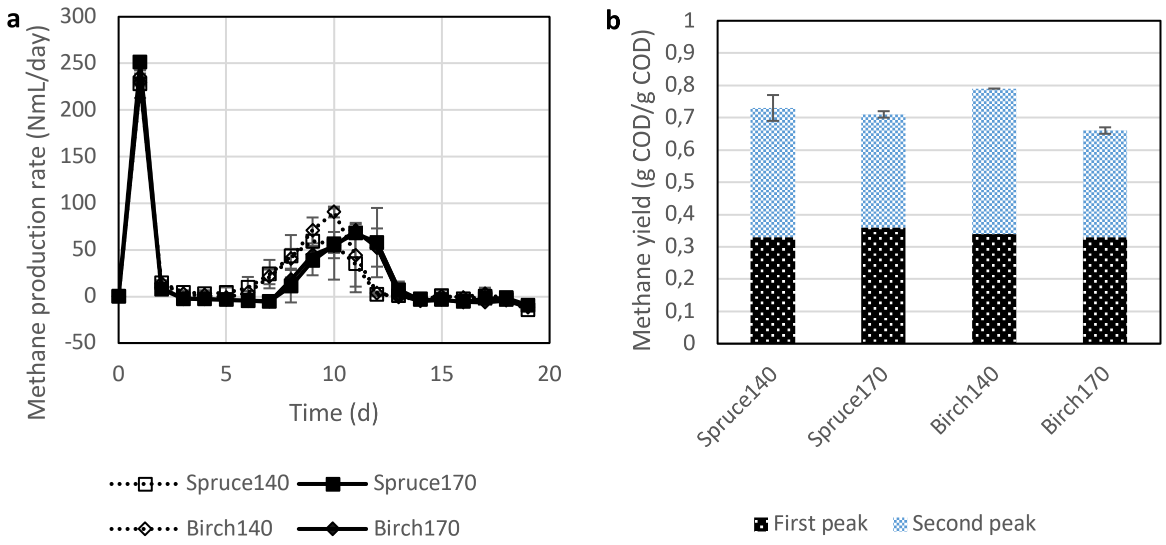


Figure 4. Methane rate and yield from hydrolysates of Norway spruce and birch HWE pretreated at 140 °C and 170 °C.

3.3.2 AD of aqueous pyrolysis liquid (APL)

The methane production from the AD reactors fed APL from pyrolysis of hot water extracted Norway spruce and birch biomass was monitored for 24 days (Figure 5a and b). Equal methane yield of around 50 % of theoretical achievable was found in all cases irrespective of types of biomass and hot water extraction pretreatment temperatures before pyrolysis. The methane yield was found to be 0.49 g COD/g COD (170.5 NmL/g COD) and 0.45 g COD/g COD (159.1 NmL/g COD) for Norway spruce and 0.49 g COD/g COD (170.7 NmL/g COD) and 0.52 g COD/g COD (181.0 NmL/g COD) for birch APL from biomass HWE pretreatment temperatures at 140 °C and 170 °C respectively.

Although the HWE temperature before pyrolysis and type of biomass had no significant effect on the methane yield, the effect on the methane production rate and the digestion time was distinct (Figure 5a). The digestion period was longer in the case of APL from hot water extracted biomass at low temperature. APL from low extraction temperature had digestion period of 21 and 24 days compared to only 17 and 19 days of Norway spruce and birch respectively extracted at high temperature. At low hot water extraction temperature, a lower amount of hemicellulose and lignin was extracted from the biomass. This might result in increased concentration of furanic compounds and acetic acid and phenolic compounds in the APL during pyrolysis and can explain the longer digestion period (Kumar et al., 2020).

The type of biomass also influenced the digestion behavior. Unlike Norway spruce, the lag phase of birch was influenced by the HWE pretreatment temperature. Higher concentration of hemicellulose in birch leads to higher hemicellulose in solid residue during low pretreatment temperature resulting in APL with higher concentration of furfural and HMF hampering the AD process (Ghasimi et al., 2016; Kumar et al., 2020). Longer lag phase in birch is also attributed to complex lignin structure of hardwood, resulting in lower dissolution of lignin for hardwood during pretreatment than for softwood, giving higher lignin concentration in solid residue, ending up in APL as lignin derivative compounds such as phenol and cresol during pyrolysis which has inhibitory nature to AD microorganisms (Fahmi et al., 2008; Kan et al., 2017; Liaw et al., 2015), and can be observed as higher undigested CODs (4.9 ± 0.04 mg/L and 4.3 ± 0.2 g/L for low and high pretreatment temperature respectively) in the digestate for birch than for Norway spruce (4.0 ± 0.2 mg/L and 3.4 ± 0.02 mg/L for low and high pretreatment temperature respectively). Anaerobic microbes can however adapt to these inhibitors over time as also suggested by the lag phase, and break down such inhibitors into methane if the concentration is not too high to permanently sour the system (Appels et al., 2011; Boopathy, 2009; Si et al., 2018).

The resulting methane yield values are in the same range as reported by others for agricultural residues (Hubner and Mumme, 2015), but considering the use here of undiluted APL, non-acclimatized inoculum and avoiding any additives to enhance the AD process, the methane yield has a potential to be even higher despite woody biomass having higher concentration of AD inhibitors in APL compared to agricultural biomass (Yang et al., 2014).

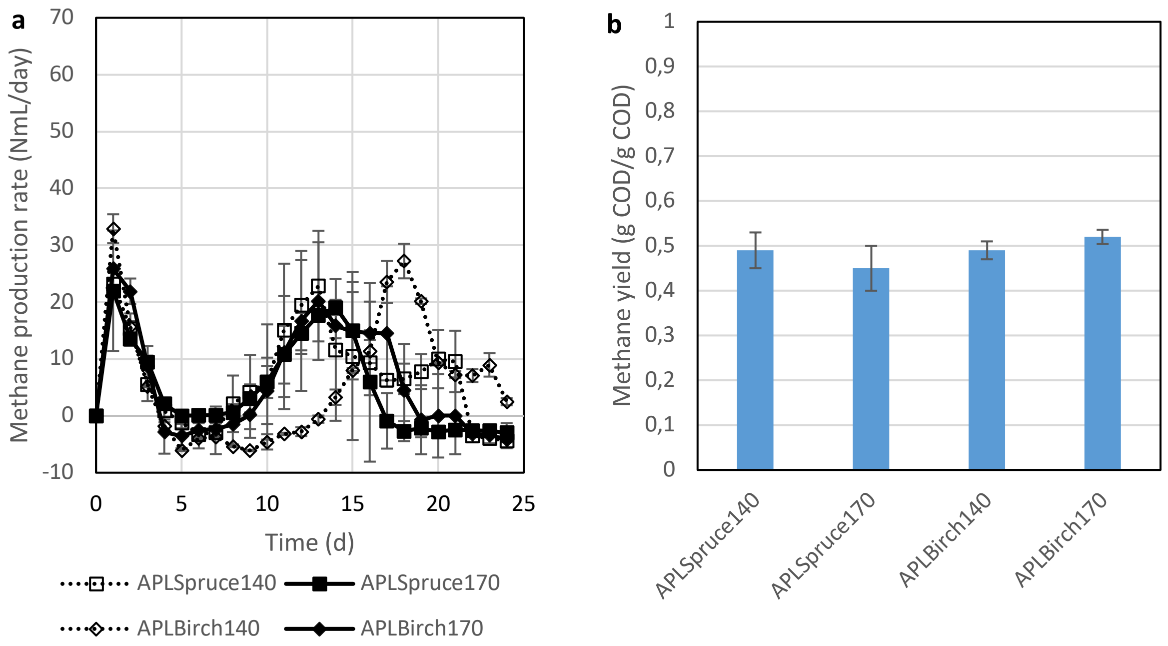


Figure 5. Methane yield and rate from AD of APL from pyrolysis (400°C) of hot water extracted Norway spruce and birch biomass pretreated at 140°C and 170°C.

3.3.3 Anaerobic co-digestion of APL with hot water extract hydrolysate

APL is co-digested with the corresponding HWE hydrolysate in two different COD based ratios to study the effect on methane yield and rate. During the co-digestion ratio (Hydrolysate:APL) of 1:1 (COD:COD), the methane yield in all the samples was found to be significantly lower ($P < 0.05$) than the sum yield from the individual substrates (APL and hydrolysate) (Figure 6a). A co-digestion ratio of 3:1 (Hydrolysate:APL) significantly ($P < 0.05$) improved the methane yield by 40 % and 6 % in Norway spruce and by 26 % and 59 % in birch pretreated at 140 °C and 170 °C respectively compared to the 1:1 ratio cases. At the 3:1 ratio the yield was not significantly different ($P > 0.05$) than the sum yield of individual substrates (Figure 6b). Diluting APL with a higher amount of sugar rich hydrolysate overcoming toxicity and inhibition of APL is also observed to give a reduced digestion period, an absence of lag phase (Figure 6c and d) and lower undigested CODs concentrations (22 % and 34 % lower for Norway spruce and 15 % and 13 % for birch for low and high pretreatment temperature respectively) at the end of the experiment during co-digestion ratio of 3:1 compared to 1:1. Similarly, regression analysis showed that the relationship between methane yield and volume of APL was relatively strong ($R^2 = 0.73$) implying that the amount of APL should be carefully controlled for enhanced methane yield during the co-digestion.

Adding APL only as an additive with a high co-digestion ratio (Hydrolysate:APL; 3:1 (COD:COD)) is beneficial in terms of rate and digestion time, with methane yield comparable to hydrolysate and APL used as sole AD feeds. As the APL production volume is not as big as that of hydrolysate in the proposed cascade process, using APL only as an additive is very practical considering the volume of feeds, toxicity of APL and easy biodegradation of hydrolysate.

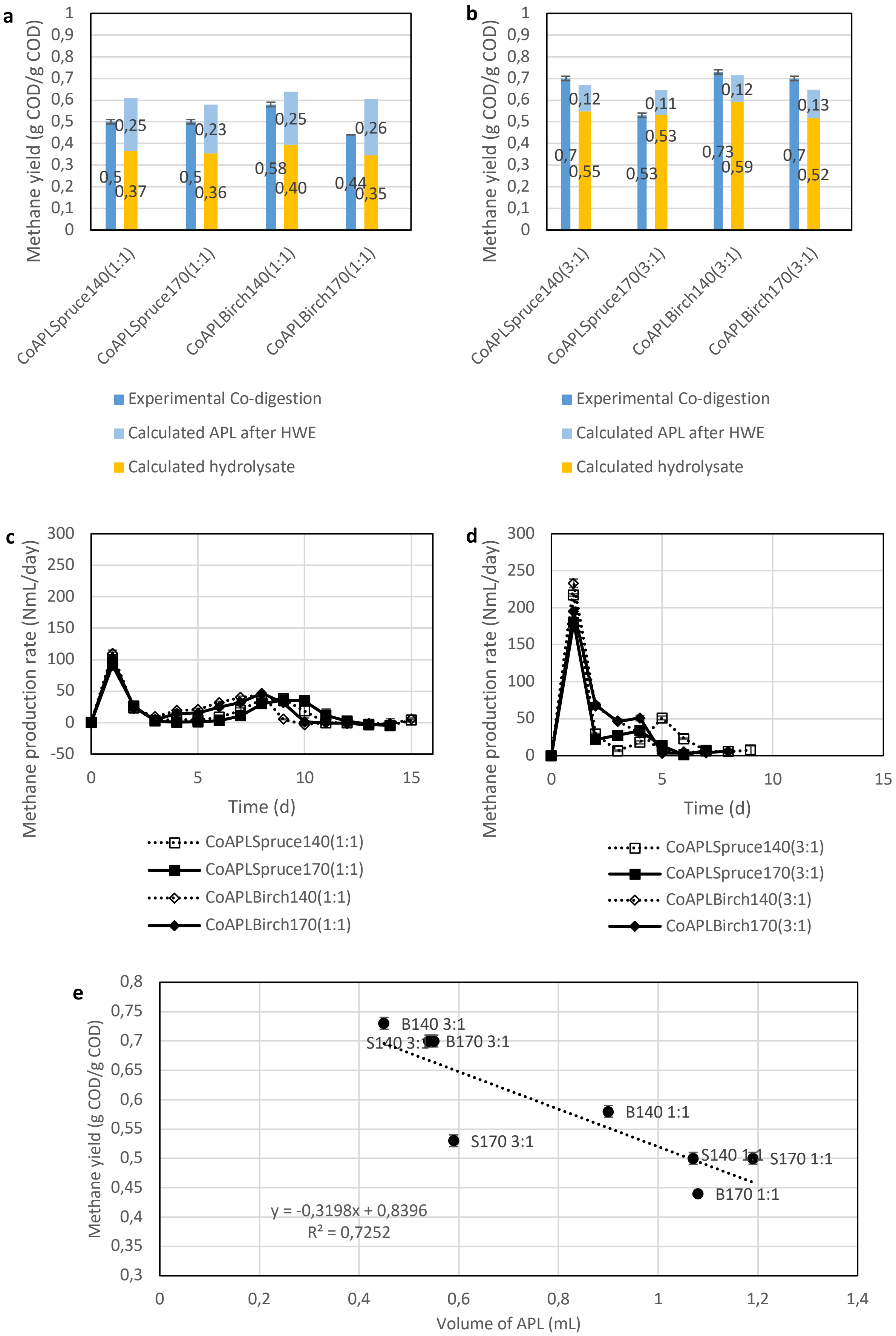

a
Methane yield (g COD/g COD)
1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
0,5
0,25
0,37
0,5
0,23
0,36
0,58
0,25
0,40
0,44
0,26
0,35
CoAPLSpruce140(1:1)
CoAPLSpruce170(1:1)
CoAPLBirch140(1:1)
CoAPLBirch170(1:1)
Experimental Co-digestion
Calculated APL after HWE
Calculated hydrolysate
b
Methane yield (g COD/g COD)
1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
0,7
0,12
0,55
0,53
0,11
0,53
0,73
0,12
0,59
0,7
0,13
0,52
CoAPLSpruce140(3:1)
CoAPLSpruce170(3:1)
CoAPLBirch140(3:1)
CoAPLBirch170(3:1)
Experimental Co-digestion
Calculated APL after HWE
Calculated hydrolysate
c
Methane production rate (NmL/day)
300
250
200
150
100
50
0
-50
0
5
10
15
Time (d)
CoAPLSpruce140(1:1)
CoAPLSpruce170(1:1)
CoAPLBirch140(1:1)
CoAPLBirch170(1:1)
d
Methane production rate (NmL/day)
300
250
200
150
100
50
0
0
5
10
15
Time (d)
CoAPLSpruce140(3:1)
CoAPLSpruce170(3:1)
CoAPLBirch140(3:1)
CoAPLBirch170(3:1)
e
Methane yield (g COD/g COD)
0,8
0,75
0,7
0,65
0,6
0,55
0,5
0,45
0,4
0,35
0,3
B140 3:1
S140 3:1
B170 3:1
B140 1:1
S170 3:1
S140 1:1
S170 1:1
B170 1:1
y = -0,3198x + 0,8396
R² = 0,7252
0
0,2
0,4
0,6
0,8
1
1,2
1,4
Volume of APL (mL)

Figure 6. (a) and (b) Methane yield, (c) and (d) methane production rate during the co-digestion of APL and hot water extract hydrolysate at different ratios and (e) variation of methane yield with amount of APL during co-digestion.

4 Conclusions

Innovative cascade processing of Norway spruce (softwood) and birch (hardwood) combining hot water extraction (170 °C for 90 minutes (severity 4.02) and 140 °C for 300 minutes (severity 3.65)), intermediate pyrolysis (400 °C) and anaerobic digestion for methane production is presented, evaluated and found to be a promising approach.

Anaerobic digestion was found to be an effective method for methane production from organic rich waste streams of hydrolysate and APL as feedstock with biodegradability of around 70 % and 50 %, respectively. Hydrolysate prepared at 140 °C temperature had better methane yield than 170 °C for birch while the pretreatment temperature did not significantly influence the hydrolysate methane yield for Norway spruce. Effect of HWE temperature on methane yield of APL was low, but it had some effect on production rate since HWE at 140 °C resulted in longer digestion time of the corresponding APL compared to 170 °C for both biomass types.

Co-digestion of APL with hydrolysate improved the overall methane yield at the COD based feed ratio of 3:1 (hydrolysate:APL) compared to the ratio of 1:1, while the improvement was not significant compared to individual digestion. This suggests that the use of APL as an additive feed in moderate ratios to hydrolysate in AD can be advantageous considering the volume of the feeds typically available in the process scheme investigated, the toxicity of the APL and the easy bio-degradation of the hydrolysate.

Declaration of Competing Interest

The authors declare no conflict of interests.

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Article 5

Liquefaction of Lignocellulosic Biomass for Methane Production: A Review

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Liquefaction of Lignocellulosic Biomass for Methane Production: A Review

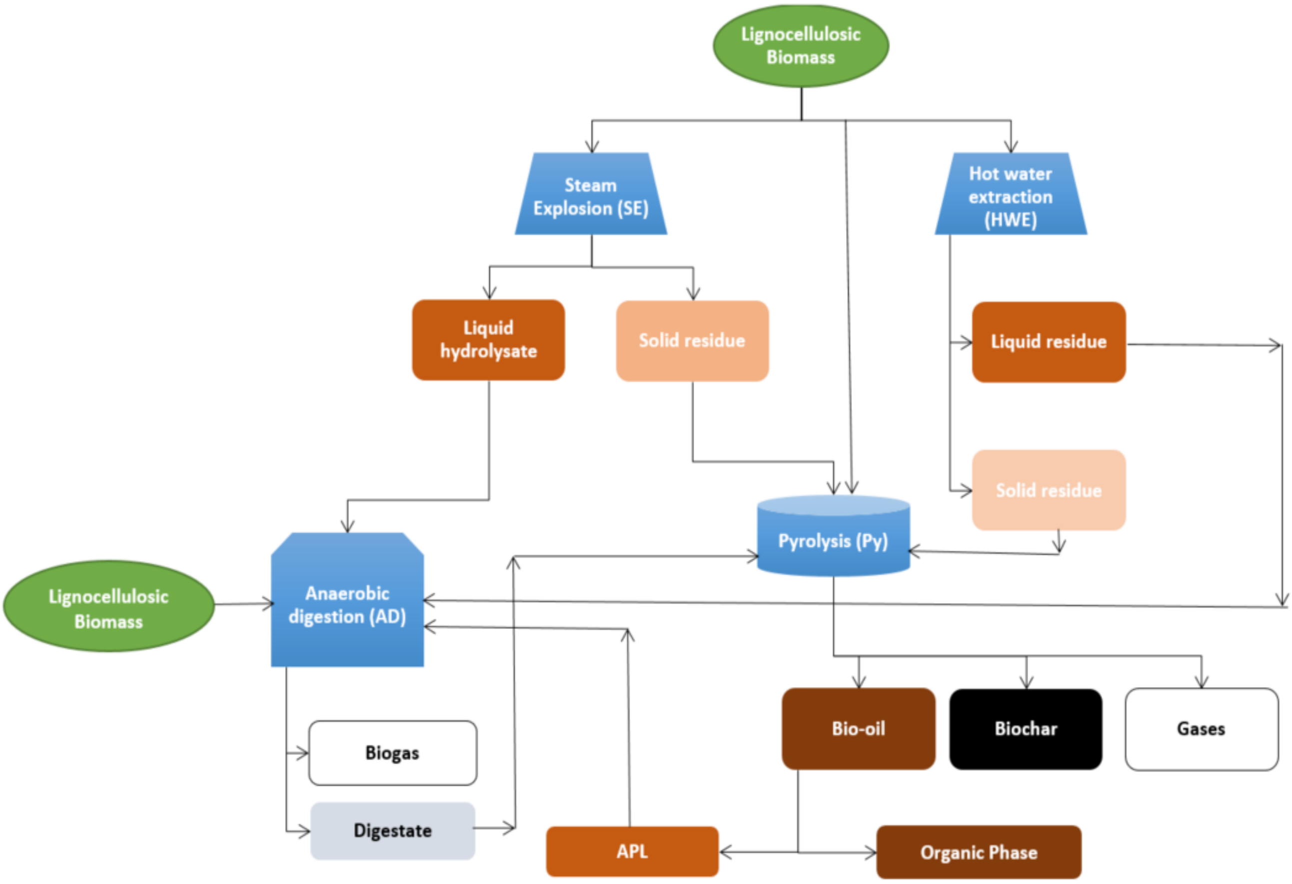
Highlights

- Liquefaction of lignocellulosic biomass by pretreatment for efficient AD is focused
- Anaerobic digestion of hydrolysate and APL from pyrolysis is reported
- Effects of inhibitors in hydrolysate and APL are discussed
- Pretreatment tuning to increase methane yield is discussed

Abstract

Hydrothermal pretreatment (HTP) (Hot water extraction (HWE) and steam pretreatment) and pyrolysis have the potential to liquefy lignocellulosic biomass. HTP produces hydrolysate consisting mainly of solubilized hemicellulose while pyrolysis produces aqueous pyrolysis liquid (APL). The liquid products either as main products or by-product, can be used as AD feeds, overcoming shortcomings of solid-state AD (SS-AD). This paper reviews HWE, steam pretreatment and pyrolysis pretreatment methods used to liquefy lignocellulosic biomass, AD of liquefied products, effects of inhibition from intermediate by-products such as furan and phenolic compounds and pretreatment tuning to increase methane yield. HTP is carried out at moderate temperatures when methane production is the main aim to reduce formation of inhibitory compounds. APL is a challenging substance for AD due to its complexity, including various inhibitory substances. However, AD microbial consortia appear capable to adapt to most of the complex compounds present in APL.

Graphical Abstract



Keywords

Lignocellulosic Biomass, Liquefaction, Hydrothermal pretreatment, Pyrolysis, Liquid state anaerobic digestion

1 Introduction

Lignocellulosic biomass is recognized as the most abundant source of renewable energy globally, available in the forms of softwood, hardwood, energy crops, grasses and agricultural residues (Nitsos et al., 2013). Bioethanol, biodiesel and biogas are biofuel products from lignocellulosic material with increasing demands. Large amount of cellulose and hemicellulose makes lignocellulosic biomass attractive for biogas production through AD (He et al., 2015). Biogas from anaerobic digestion (AD) is an environmentally friendly, widely accepted and highly promising bioenergy alternative to fossil-derived energy (Sawatdeenarunat et al., 2015). Lignocellulosic biomass also has competitive advantages over other AD feedstock due to their abundance, low price, relatively high yield and non-interference with food supply (Dahadha et al., 2017). It can however vary to what extent these feedstocks can be treated in sustainable ways by AD to produce biogas. A wide range of lignocellulosic biomass has been considered as feeds for AD.

However, lignocellulosic biomass is not limited to only energy production as it has historically been used for direct combustion or methane production by SS-AD after simple pretreatments such as milling. With the invention of new technologies and methods, it has been considered for various new products. Recently, cascade utilization of lignocellulosic biomass is considered to obtain maximum utilization that would make biofuels more economically competitive with fossil fuels (Rasi et al., 2019). This approach brings forest-based lignocellulosic biomass into a domain of AD where it can be applied in conjunction with producing several energy carrier products, including biochar and bio-oils, and high value chemicals (Rasi et al., 2019). Similarly, lignocellulosic biomass is pretreated by hydrothermal methods to make it more favorable for subsequent use such as to improve mechanical strength of the solid biomass based material (Alvarez-Chavez et al., 2019). During these processes of thermal pretreatment, hemicellulosic sugar-rich liquid with some dissolved lignin, commonly called hydrolysate, is released during hydrothermal pretreatment and aqueous pyrolysis liquid (APL) during pyrolysis as by-products (Liu, 2015). It is important to utilize these organic rich liquid streams. Hydrolysate is pentose rich and not considered suitable for bioethanol production due to requirement of genetically modified microorganisms thriving on pentose (Kaparaju et al., 2009b) and high sensitivity to inhibitors like furfural and hydroxyl methyl furfural (5-HMF) present in the hydrolysate (Torry-Smith et al., 2003). It has also been previously considered for production of hydrogen through dark fermentation, production of health food additive, acetic acid, ethanol, butanol, lactic acid and polyhydroxyalkanoates (PHA), but the results are not promising (Liu, 2015). Similarly, APL is a challenging substrate with various toxic compounds, which can be handled only with robust consortia of microorganisms. Therefore, AD could be a good choice to exploit and handle the organic rich liquid by-product stream to produce methane.

AD is a biological process where a mixed community of microorganisms act together to break down organic compounds to produce biogas (about 50-75 % CH_4 and 25-50 % CO_2) in the absence of free oxygen. An advantage of such consortia of microorganisms is their ability to synergistically break down various complex, recalcitrant and inhibiting compounds (in low concentration) to methane after some adaptation time (Benjamin et al., 1984).

Reported AD of pretreated lignocellulosic biomass is mostly solid state AD (SS-AD) which is operated at a total solid (TS) content of 15 % or higher, contrary to liquid-state AD (L-AD) that operates at TS content of less than 15 % (Guendouz et al., 2010). SS-AD has several advantages but also several inherent problems. Some of the well noted problems are low methane yield, slow mass transfer between AD microorganisms and feedstock, potential instability, acidification, ammonia inhibition, nutrient imbalance, temperature disturbance and obstacles in using end products (Yang et al., 2015). Several methods have been proposed to overcome these issues (Xu et al., 2019). Liquefaction of lignocellulosic biomass is the most disruptive approach as it changes the inlet mode of lignocellulosic biomass from solid to liquid so that a wider range of AD reactors, including high rate sludge bed, can be applied. Review of L-AD of liquefied lignocellulosic biomass, however, is still scarce although a notable number of research papers on such solutions are published.

This review aims to encapsulate research related to L-AD of thermally pretreated lignocellulosic biomass, without addition of chemicals, where AD can both be a method to treat by-products from utilization of lignocellulosic biomass for other purposes or being the main process. The recalcitrant composition of lignocellulosic biomass is first presented, followed by constrains and challenges of AD of lignocellulosic biomass. Next, different thermal pretreatment methods which overcome recalcitrance by liquefying the lignocellulosic biomass is elucidated. Both the favorable and unfavorable products formed during the pretreatments are discussed followed by their effects on AD. Optimization of pretreatments to increased AD yield is discussed before concluding remarks.

2 Lignocellulosic Biomass as feed for AD

The composition of lignocellulosic biomass creates constrains and challenges for direct AD on the lignocellulosic biomass.

2.1 The composition of lignocellulosic biomass

Lignocellulosic materials are primarily composed of three types of polymers: cellulose (30-50 %), hemicellulose (15-30 %) and lignin (10-20 %) (Jørgensen et al., 2007). Cellulose and hemicellulose are chain polysaccharides, while lignin, closely associated with cellulose and hemicellulose, is a heterogeneous, phenolic polymer (Mussatto et al., 2008) (Figure 1). The composition of lignocellulosic biomass depends on the plant species, its growth stage and environment (Surendra & Khanal, 2015). Non-structural carbohydrates like glucose, fructose, and sucrose along with proteins, lipids, and pectin are also present in different concentrations in the lignocellulosic materials (McDonald et al., 1991).

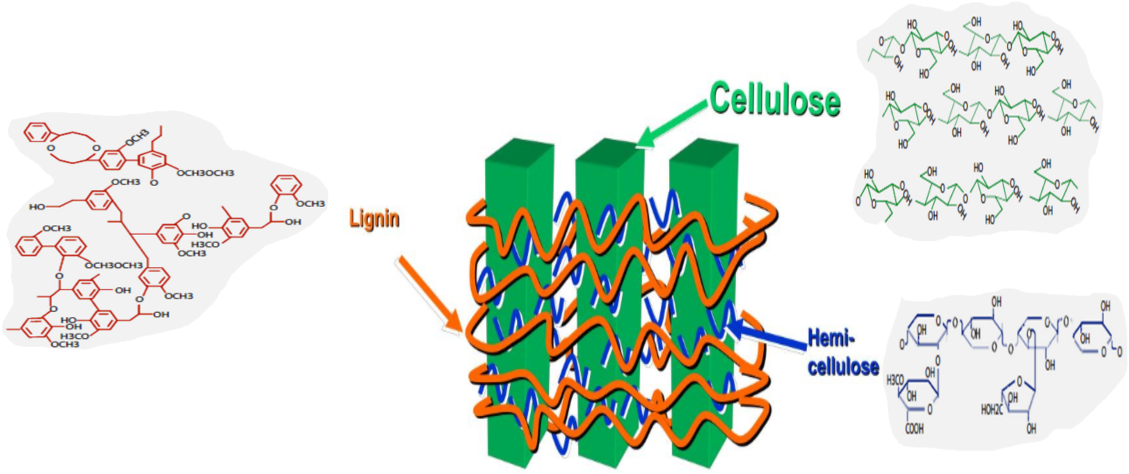


Figure 1: Structural composition of lignocellulosic biomass (Adapted from (Alonso et al., 2012)).

2.2 Constrains and challenges for AD of lignocellulosic biomass

Lignocellulosic biomass can be treated anaerobically to produce biogas. However, hydrolysis, the rate-limiting step for lignocellulosic material that breaks down the complex organic polymer components during AD, is a very slow process for lignocellulosic materials and is sensitive to the type and composition of the substrate (Dahadha et al., 2017).

Other constrains to the AD process is the heterogeneity and low density of lignocellulosic substances that hampers AD by forming a floating layer on the surface of AD reactors (Tian et al., 2015). This leads to poor substrate accessibility for the microorganisms, worsening mass and heat transfer and reducing the methane yield (Wang et al., 2018). High C/N ratio can also limit AD of lignocellulosic biomass (Sawatdeenarunat et al., 2015) but it can be adjusted by adding nitrogen sources (at added cost) or co-digesting with substrate with higher nitrogen content. These extra costs can be offset by increased biogas production to make lignocellulosic biomass a lucrative AD feed for methane production.

3 Pretreatment methods

Pretreatments can be categorized into different groups such as mechanical or physical, thermal, hydrothermal, chemical and biological. Each pretreatment method works differently, resulting in different yields and products. A single pretreatment cannot necessarily be recommended for each feedstock as their compositions vary considerably and pretreatment at the same operational conditions would therefore behave differently for the same feedstock (Ahmad et al., 2018). Pretreatments can be applied alone or combined and should be chosen based on feedstock and the desired final products (Pelaez-Samaniego et al., 2013). Readers are referred elsewhere to a comprehensive review for an in-depth understanding of aforementioned pretreatments (Zheng et al., 2014).

Pretreatment typically accounts for 30 % of the total cost of a biofuel (Alonso et al., 2013) and it has to be cost effective to be applied at large scale (Di Girolamo et al., 2013). Pretreatment involving chemical tends to be costly both due to added costs of chemicals and due to added cost of handling chemical waste streams and are therefore not included here. Only the liquefying pretreatments hydrothermal (example; steam pretreatment and hot water extraction) and pyrolysis are covered in this paper.

3.1 Hydrothermal

Hydrothermal pretreatment is the most common pretreatment to convert lignocellulosic biomass into biofuel or other valuable products (Di Girolamo et al., 2013). Hydrothermal is commonly defined as “a reaction occurring under the conditions of high temperature and pressure in aqueous solutions in a closed system” (Rabemanolontsoa & Saka, 2016). Hydrothermal temperature and pressure are maintained under the critical point (374 °C and 22.1 MPa) for water (He et al., 2015). The hydrothermal process does not need washing, chemical recovery or detoxification steps (Sun et al., 2014), and additional advantages like no catalyst requirement, low reactor cost and direct utilization of wet or fresh lignocellulosic materials make hydrothermal pretreatment very attractive (Batista et al., 2019; Sun et al., 2016). The process can also have limitations that must be considered before industrial implementation such as difficult tasks of separation and extraction of different streams at industrial scale (Ahmad et al., 2018). Only two primary hydrothermal treatments, viz. hot water extraction and steam pretreatment, with or without explosion, for biogas production are found and discussed in this paper.

Several review articles are available on hydrothermal treatment of lignocellulosic biomass (Elliott et al., 2015). However, as per our knowledge, only two review articles are available on hydrothermal pretreatment of lignocellulosic biomass especially for AD (Ahmad et al., 2018; He et al., 2015). The review by Ahmad et al. (2018) delivers deep insights into effects on structural components and techno-economic analysis coupled life cycle assessment (LCA). The review by He et al. (2015) covers operating conditions of hydrothermal treatment for subsequent biogas production.

3.1.1 Hot water extraction

Hot water extraction (HWE) (also called liquid hot water (LHW) or hydrothermolysis) is a mild hydrothermal pretreatment carried out in the temperature range 120-230˚C and at various pressure conditions at which water is kept in subcritical conditions (Nitsos et al., 2013). Its objective is to efficiently extract sugars, acids, or other chemicals from lignocellulosic biomass without considering any structural changes in the extracted wood (Pelaez-Samaniego et al., 2013). Sometimes, the term hot water extraction is used for milder condition while liquid hot water pretreatment is used for harsh condition (in terms of temperature and pressure). They are not differentiated as such in this review.

3.1.2 Steam pretreatment/steam explosion

Steam pretreatment can be performed in two different ways; (i) without addition of other chemicals as catalysts, also called 'autohydrolysis' and; (ii) with additional of other chemicals like acid, alkali or supercritical catalysts to reduce process severity, called 'catalyzed steam-pretreatment'. During autohydrolysis, which is discussed here, acetyl residues from xylan hemicellulose are liberated as acetic acid and catalyze the reaction (Rabemanolontsoa & Saka, 2016). To prevent acetic acid loss by evaporation, low pH and high temperature should be avoided (Bruni et al., 2010).

Steam pretreatment and steam explosion pretreatment involves high temperature heating where the difference between the two methods is that at the end of steam explosion, water in the biomass explode due to the rapid depressurization and cooling down of the biomass to disrupt the biomass fibers (Hendriks & Zeeman, 2009). Typical ranges for temperature, pressure and time are 160-260˚C, 0.69-4.83 MPa and several seconds to a few minutes, respectively (Sun & Cheng, 2002). Steam explosion is one of the most efficient hydrothermal physical or physio-chemical pre-treatment methods for recalcitrance disruption of lignocellulosic biomass (Wyman et al., 2005). It has been found that steam explosion saves almost 70 % energy compared to conventional mechanical process to attain the same particle size (Holtzapple et al., 1989). Its earliest patent is dated to 1924 although it is known since the 1800s (Rabemanolontsoa & Saka, 2016). Nowadays, it is commonly used for pretreatment of woody biomass (Vivekanand et al., 2013) and agricultural residues (Zhao et al., 2018). These pretreatments are claimed to have good environmental influence, low hazardous chemicals yields and high-energy efficiency (Bhutto et al., 2017; Yan et al., 2016) while disadvantages for steam pretreatment/steam explosion may include high water demand and expensive equipment (Sun et al., 2016; Zheng et al., 2014).

In this article, the terms steam pretreatment and steam explosion pretreatment are used interchangeably since they have the same objective of producing sugar rich feed for AD process. However, they should be treated independently if change in structure of biomass is to be studied.

3.2 Pyrolysis

Pyrolysis breaks down chemical bonds to form new compounds in the absence of oxygen and has a high flexibility in processing raw biomass materials for derived end products (Rasi et al., 2019). It converts biomass thermo-chemically into liquid (bio-oil and also called pyrolysis oil, pyrolysis liquid, bio-crude, wood liquid, wood oil or wood distillate), charcoal (biochar) and non-condensable gases (syngas) by heating to about 480 °C or more (Pecchi & Baratieri, 2019). Bio-oil consists of molecules derived from the degradation of cellulose, hemicellulose and lignin (Alvarez-Chavez et al., 2019). Higher cellulose content leads to higher liquid products, high hemicellulose content leads to higher gas production and higher lignin content leads to more solid residues (Kumar et al., 2020). Due to the presence of high concentration of water in the feedstocks, the bio-oil is separated into aqueous phase (APL) and organic phase. The organic phase (or biocrude) is a complex mixture of oxygenated hydrocarbons and nitrogenated compounds such as aromatics, short chain carboxylic acids, ketones, phenolics, sugars and derivatives of furan depending upon the type of biomass (Hassan el et al., 2009). Its complex nature together with high oxygen levels makes it difficult to utilize this organic phase directly in AD, and it can enter the market as a renewable alternative to heavy fuel oil (Oasmaa et al., 2015). APL, on the other hand, has a high chemical oxygen demand (COD) concentration along with various potentially toxic organic compounds and can be environmentally harmful if not managed properly (Seyedi et al., 2019).

Process parameter adjustments varies the proportion of produced bio-oil, biochar and syngas. Lower process temperature and longer vapor residence time favors char production. High temperature and longer residence time increases syngas formation, while moderate temperature and short vapor residence time produces optimum bio-oil (Bridgwater, 2012). Pyrolysis is differentiated into four main categories based on residence time, slow (or conventional), moderate (or intermediate), fast and flash (Table 1) (Bridgwater, 2012; Giwa et al., 2019). In fast pyrolysis, liquid with only a single phase is obtained (Oasmaa et al., 2015) while intermediate pyrolysis produces two phase liquid (organic phase and aqueous phase (APL)) (Fabbri & Torri, 2016). Details of these processes can be found elsewhere (Kan et al., 2016).

Table 1: Different modes of pyrolysis technologies, corresponding process conditions and typical product weight yield.

Mode	Conditions		Products		
	Temperature (˚C)	Residence time	Liquid (%)	Char (%)	Gas (%)
Fast	~500	~1 s	75	12	13
Moderate	~500	~10-30 s	50	20-25	25-30
Slow	~500	~5-30 min	30	35	35
Flash	400-950	30 ms-1.5 s	70	25	16

4 What can be achieved during pretreatment?

Pretreatment can efficiently deal with the slow hydrolysis of lignocellulosic materials by disrupting the lignin barrier, solubilizing the hemicellulose, reducing the cellulose crystallinity, increasing the surface for enzymatic attack and homogenizing the lignocellulosic biomass to improve biodegradability (Bhatia et al., 2020; Di Girolamo et al., 2013; Sawatdeenarunat et al., 2015). However, pretreatment is not limited to improve biodegradability but can also be carried out for conditioning woody biomass to optimize production of other products such as biochar, bio-oil and valuable products (Alvarez-Chavez et al., 2019; Pelaez-Samaniego et al., 2013; Rasi et al., 2019). According to these studies such processes may generate biproducts that can be handled by AD but in depth evaluations of such solutions are lacking. The products from hydrothermal treatment and pyrolysis are results of different temperatures, pressures and water contents, forming a vast variety of components, from easily degradable to inhibiting.

4.1 Wanted effects and products related to AD

4.1.1 Hydrothermal

During hydrothermal pretreatment, water or steam (or both) penetrates under high pressure (with or without catalysts) to liberate most of the hemicellulose and partial lignin while making biomass more accessible to hydrolytic enzymes (Taherzadeh & Karimi, 2008). At pretreatment temperature of 100°C, hemicellulose remains in the solid fraction but at temperature above 150°C, hemicellulose hydrolyzes and dissolves into the liquid fraction (or hydrolysate) (Fernandez-Cegri et al., 2012; Hendriks & Zeeman, 2009). At temperature 150-180°C, solubilization of firstly hemicellulose and shortly after lignin starts (Hendriks & Zeeman, 2009). Cellulose begins decomposing at 200°C with reaction rate faster at higher temperature (> 240°C) and the reaction completing at around 280°C (Minowa et al., 1997).

Aqueous hemicellulose (or the extract) consists mainly of oligomeric and monomeric products like xylose, acetyl, glucose, mannose, rhamnose, arabinose and galactose (Mosier et al., 2005b). In the case of AD, hydrothermal pretreatment should liberate hemicellulosic organic compounds utilized by microorganism such as pentose (xylose and arabinose), hexose (mannose, galactose, glucose), volatile fatty acids (VFA), proteins and lipids (Bruni et al., 2010). The composition of aqueous hemicellulose depends on the sources of lignocellulosic biomass and the extraction time. The major carbohydrate in the hydrolysate of e.g. sugar maple (hardwood) woodchips is xylose, with minor other sugars (Liu, 2015), but at short extraction time (around 60 minutes only) glucose and mannose concentrations are higher than xylose (Liu, 2013). Unlike hardwood, southern pine (softwood) has mannose as the dominant sugar species followed by other sugars in the hydrolysate (Liu, 2015). Part of the hemicellulose is hydrolyzed and form acids during hydrothermal processing. Acetic acid is the most abundant VFA produced while other VFAs are found to be negligible (Di Girolamo et al., 2013). The produced acetic acid acts as a catalyst during the process in degrading the polymers and thereby contributes in increasing the sugar yield (Mosier et al., 2005b).

In recent years, there is a significant increase of research using hydrothermal pretreatment for biogas production. Agricultural residues, which are increasingly becoming an issue of waste handling, are subjected to hydrothermal pretreatment to make them AD favorable for efficient utilization. Hydrothermal pretreatment can also treat lignocellulose previously considered unsuitable for AD, such as wood to remove hemicellulose and lignin (to some extent), alter the crystallinity index of cellulose or reduce the degree of polymerization of cellulose and lignin (Pelaez-Samaniego et al., 2013). Hydrothermal pretreatment also acts as pretreatment to lignocellulosic biomass to make a suitable feedstock for processes such as pyrolysis to produce biochar (Figure 2), a product with multiple uses such as natural fertilizer or solid fuel (Feng & Lin, 2017). It has also been found as an additive to AD for enhanced methane production (Torri & Fabbri, 2014).

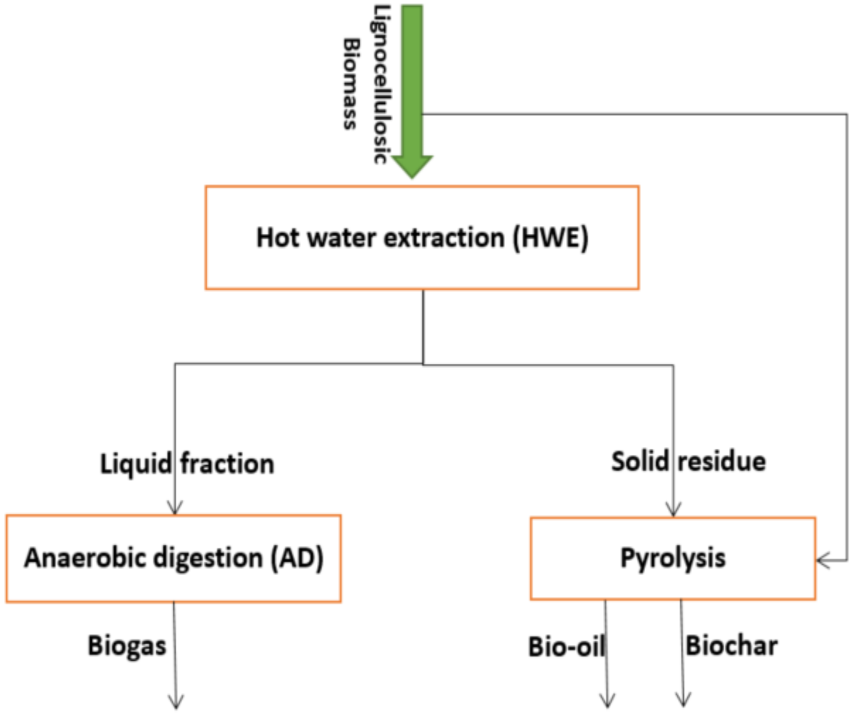


Figure 2: Flow chart of biogas and bio-oil/biochar production from liquid fraction and solid fraction, respectively, of lignocellulosic biomass after hydrothermal pretreatment.

4.1.1.1 Hot water extraction

During hot water extraction, part of the hemicellulose and some lignin are dissolved in the extraction water liquor (Hendriks & Zeeman, 2009). Cellulose cannot be dissolved during this process since concentrated acid or higher temperature is required (Monlau et al., 2013). This leads to relative increase in cellulose and lignin contents in the woody biomass as hemicellulose is removed. This has positive effects on the quality of the solid biomass products like composites and various board products made, such as reduced water absorption, improved mechanical properties and improved resistance to decay (Pelaez-Samaniego et al., 2013). In case of using solid residues after hot water extraction for ethanol and biogas production, hydrothermal pretreatment enhances the yield as glucose (from cellulose) is better converted without the presence of mixed sugars (Liu, 2015) and cellulose mostly remains in the solid residue in a loose form for easier hydrolysis. Similarly, hydrothermal pretreatment is also in use to make cellulose better accessible for enzymatic fermentation in subsequent bioethanol production (Mosier et al., 2005b). HWE before pyrolysis also gives better bio-oil yield due to increased cellulose content while reducing ketones, acids and water content in the bio-oil leading to higher heating value and significantly improved levoglucosan content (Chang et al., 2013). HWE also lowers the activation energy for thermal degradation during pyrolysis compared to untreated biomass (Kumar et al., 2020).

HWE has been successfully used in pre-treating agricultural residues to make them AD suitable (Baeta et al., 2016a; Luo et al., 2019). However, reported HWE of woody biomass is limited mostly to make woody biomass rich in cellulose and lignin by removing hemicellulose (Pelaez-Samaniego et al., 2013), not considering AD for methane production.

4.1.1.2 Steam pretreatment

The objective of both steam pretreatment and steam explosion pretreatment is to obtain solubilized hemicellulose (Bruni et al., 2010; Teghammar et al., 2010), but also some lignin will be removed from the lignocellulosic materials during both pretreatments (Pan et al., 2005). During steam explosion, pressure is reduced rapidly to atmospheric condition to disintegrate the biomass partly or completely decreasing the particle size, depending upon the type of biomass (Table 2) (Rabemanolontsoa & Saka, 2016). This also increases the cellulose fiber reactivity of the residues, as the cellulose is made easily accessible for the enzymes (Laser et al., 2002). It can be of benefit if residues are also considered for bioprocesses such as biogas or ethanol production but at the expense of biochar yield.

Table 2: Effects of hot water extraction and steam pretreatment on lignocellulosic biomass (adapted from (Hendriks & Zeeman, 2009; Sun et al., 2016; Zheng et al., 2014)).

Components	Effects on the composition and structure	Effects of hot water extraction (HWE)	Effects of steam pretreatment
Cellulose	Increase contact surface area	High	High
	Reduce particle size		High
	Reduce degree of polymerization	Partial	Partial
	Reduce crystallinity	Partial	Partial
	Increase solubilization	Inefficient or low	Partial
Hemicellulose	Increase solubilization	High	High
	Formation of degradation products (e.g Furfural, HMF)	Partial	High

Lignin	Structural change	Partial	High
	Increase solubilization	Inefficient or low	Partial
	Formation of degradation products (e.g, Phenol)	Inefficient or low	Inefficient or low

Steam pretreatment is also applied to modify woody biomass to increase the strength of its products by removing hemicellulose, ash and alkali metal contents (Biswas et al., 2011). Steam pretreatment also improves calorific value by enhancing carbon content and decreasing oxygen content, pellet density, impact resistance and abrasive resistance of the pellets of biomass feedstock (Kan et al., 2016). Steam pretreatment is also used as HWE in making cellulose more accessible to enzymes by removing hemicellulose and lignin for further conversion to fermentable simple sugars such as ethanol (Simangunsong et al., 2018). In these processes, high yield of hemicellulosic sugars are recovered in liquid phase consisting of monomers, oligomers, and polymers which have possibilities of AD digestion (Simangunsong et al., 2018).

4.1.2 Pyrolysis

Bio-oil from pyrolysis is a dark brown, free flowing liquor with pungent smell that consists of a complex mixture of up to 400 organic compounds such as acids, sugars, alkenes, esters, ethers and different oxygenates (Kan et al., 2017; Rezaei et al., 2014). Bio-oil can be upgraded to fuels due to the presence of phenolic monomers and dimers (Mortensen et al., 2011), its ideal carbon numbers (C_6-C_{20}) and relatively lower oxygen content compared to carbohydrate (Zhou et al., 2019). Similarly, engineered microorganisms (e.g. *Escherichia coli*) can utilize the pyrolytic sugars such as levoglucosan to produce fuels and chemicals (Layton et al., 2011). However, high water content of bio-oil poses several challenges such as low heating value and chemical and thermal instability (Zhou et al., 2019). To overcome such problems, bio-oil can be further divided into organic or oily phase (heavy fraction) and aqueous pyrolysis liquid (APL) (lighter fraction) (Figure 3). The organic or oily phase is considered as an energy source and for chemical production (Rasi et al., 2019), and it has also been considered used for bio-based pesticides (Hagner et al., 2018) and in manure acidification (Keskinen et al., 2017).

The lighter fraction of the bio-oil, APL, has a high water content and contains C_2-C_6 sugars, furan derivatives, hydroxyacids, oligomers, water soluble phenols and other water soluble organics formed during pyrolysis (Shanmugam et al., 2017). APL contains high concentration of acetic acid, ranging from 25 to 166 g/L, making it a suitable substrate for AD as acetic acid is directly converted to methane (Seyedi et al., 2020b; Wen et al., 2020). A fraction of the biochar produced can be used as effective additives for AD improving the AD reactor conditions (Torri & Fabbri, 2014).

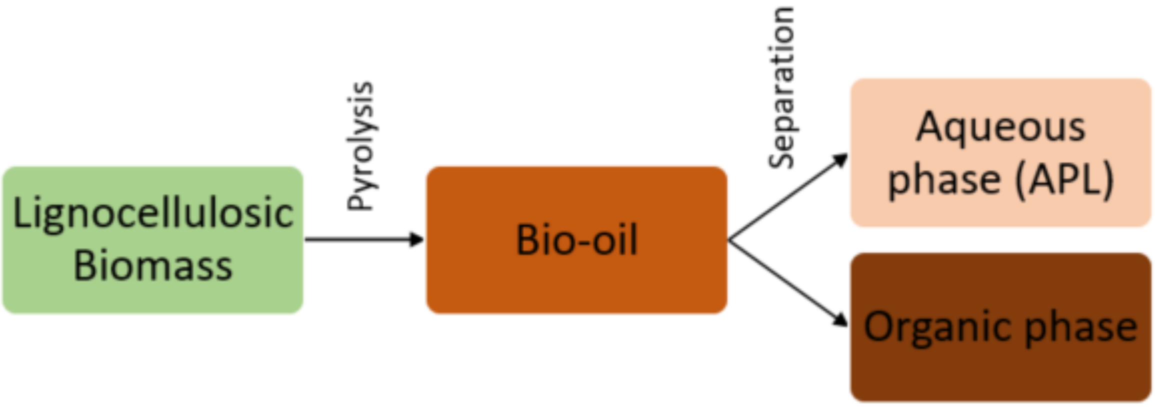


Figure 3: Flow chart of APL production from lignocellulosic biomass through pyrolysis.

The combined process of hydrothermal pretreatment and pyrolysis is of interest as it improves the bio-oil quality, for upgrading to fuel, during the subsequent use of hydrothermally pretreated feedstock in pyrolysis (Alvarez-Chavez et al., 2019). It also improves pyrolysis and gasification efficiencies by removing alkali metal responsible for slagging, corrosion and fluidized bed agglomeration while increasing energy content of the residues (Liu, 2015).

4.2 Unwanted pretreatment products related to AD

4.2.1 Hydrothermal

The objective of hydrothermal pretreatment is to achieve sufficient solubilization of the hemicellulose while limiting the formation of inhibitory compounds for increased biogas yields (Bruni et al., 2010; Teghammar et al., 2010), however, moderate formation of inhibitory products will occur (Sun et al., 2016; Zheng et al., 2014). Hydrothermal temperatures are maintained in a wide range of 160 to 240 ˚C to avoid degradation of cellulose which occurs at temperature above 240 ˚C (Cao et al., 2014). Increase in temperature during pretreatment leads to formation of AD inhibitors like furans from sugars degradation (Mosier et al., 2005a; Simangunsong et al., 2018) and soluble phenolic compounds from lignin polymer and/or lignin oligomers (Monlau et al., 2014). In addition, partial lignin degradation leads to inhibitory compounds such as vanillin and syringaldehyde (Barakat et al., 2012). Humic acids can also be inhibitory formed from the phenolic compounds (Bolyard et al., 2019; Zhang et al., 2019). These degradation products are inhibitory to bacterial fermentation and can lower the methane yield in AD (Palmqvist et al., 1999).

Aqueous hemicellulose (or the extract) which consists mainly of oligomeric and monomeric sugars have a relatively low inhibitor content in the treated samples (Mosier et al., 2005b). The furan compound 2-furaldehyde, referred to as furfural, forms due to degradation of pentose while 5-hydroxymethyl-2-furaldehyde, referred to as 5-HMF, forms because of hexose degradation (Jonsson & Martin, 2016). Less 5-HMF is formed compared to furfural due to limited hexose degradation (Chandel et al., 2011).

As part of lignin can be dissolved along with the hemicellulose (Mosier et al., 2005b), it can inhibit different steps of AD (Koyama et al., 2017). Soluble lignin itself is either inert (Liu et al., 2017) or has very low biodegradability under anaerobic condition (Benner et al., 1984), but hemicellulosic sugars, which are readily biodegradable, appear to be less biodegradable or even completely refractory when in combination with lignin (Li et al., 2018).

4.2.2 Pyrolysis

APL from pyrolysis consists of known inhibitory organic compounds such as cresol, hydroxyacetaldehyde, acetol, furans, phenols and N-containing compounds such as pyrazine and pyridine together with several compound with unknown effect (Kan et al., 2017). APL also contains inorganic constituents such as NH_3-N, H^+ or OH^-, which can reduce methanogenic activity (Seyedi et al., 2020b).

5 Thermal pretreatment AD implications

Hydrothermal pretreatment overcomes recalcitrance of lignocellulosic biomass by producing an organic rich hydrolysate. Although hydrolysates of agricultural residues and energy crops have been applied extensively in AD, an alternative use of hydrolysate from woody biomass as feed for AD is not much explored so far. The hydrolysate produced by hot water extraction and steam pretreatment of mainly agricultural residues and energy crops have been found effective in increasing the AD yield.

Similarly, from pyrolysis the lighter fraction of the bio-oil (APL) has a high water content and is rich in components that can be converted to methane by AD (Cordella et al., 2012), but with significant inhibitors present. APL from pyrolysis of lignocellulosic biomass has shown promising results as feed for AD. Combining pyrolysis and AD has the potential to increase the overall product yields from organic feedstock by overcoming defects in each individual process, and several combinations of pyrolysis and AD are therefore considered.

5.1 Hot water extraction (HWE)

HWE can be considered a mild extraction method so that concentrations of inhibitors produced are low and typically limited to furfural and 5-HMF that remains within 1.5 % of the organics (Phaiboonsilpa, 2010). The relatively high input of water during HWE implies that the solubilized lignin concentration is relatively low in the extracted liquid and precipitation of lignin compounds in AD is thus avoided (Hendriks & Zeeman, 2009). Some claim, however, that HWE based feed demands more energy for down-stream processing (AD) because of the large volume of water involved, making equipment cost high (Bhutto et al., 2017; Sun et al., 2016) but this may not be so for an optimized process.

Although L-AD study on wood (Norway spruce) as feedstock was examined recently (Ghimire et al., 2020), L-AD of woody biomass is still rare. However, L-AD of different agricultural residues (Table 3) is available and gives an enhanced methane production due to HWE pretreatment, compared to untreated, from 20 % for wheat straw (Chandra et al., 2012b) to 222 % for rice straw (Chandra et al., 2012a).

Table 3: Methane production from hot water extracted hemicellulose of different lignocellulosic biomass.

Lignocellulosic biomass	Reactor type, mode and volume	Applied pretreatment	Digestion period	Methane yield	Loading	Reference
Sugarcane bagasse	UASB, continuous, 2.3 L, 20-30 °C	178.6˚C, 43.6 min	168 days	270 L CH_4/kg COD[a]	OLR: 1.4 to 4.8 gCOD/L.d	Ribeiro et al. (2017)
Sugarcane bagasse	Two-stage (acidogenic-methanogenic), batch, 275 mL, 35 °C	185˚C, 55 min 178.6˚C, 43.6 min 178.6˚C, 55 min 182.9˚C, 40.71 mim	20 days	1 ± 0.02 Nm^3 CH_4/kg TOC 1.81 ± 0.015 Nm^3 CH_4/kg TOC 1.52 ± 0.035 Nm^3 CH_4/kg TOC 1.52 ± 0.052 Nm^3 CH_4/kg TOC	F/M ratio: 0.4 gTOC/gVSS	Baeta et al. (2016b)
Sugarcane bagasse	Batch, 275 mL, 35.1 ± 0.3 °C	170˚C, 35 min	NA	0.789 Nm^3 CH_4/kg TOC	F/M ratio: 0.4 gTOC/gVSS	Baeta et al. (2016a)

		185˚C, 35 min		0.615 Nm^3 CH_4/kg TOC		
Sugarcane bagasse	Batch, 300 mL (200 mL working volume), 35 ± 1 °C	200˚C, 10 min	30 days	198 L $CH_4/kg_{Substrate}$	OL: 1.5 gCOD/L	Costa et al. (2014)
Sugarcane press mud	Batch reactor, 500 mL, 37.5 °C	140-210 ˚C, 2-23 min	30 days	340.8 mL CH_4/g VS	S/I: 2 (based on VS)	López González et al. (2014)
Wheat straw	Semi-continuous CSTR, 5 L (3.5 L working volume), 55 °C	195˚C, 10-12 min	102 days	297 mL CH_4/gCOD	OLR: 1.9 gCOD/L.d (100 % v/v hydrolysate)	Kaparaju et al. (2009a)
	Semi-continuous UASB, 334 mL (255 mL working volume), 55 °C		90 days	267 mL CH_4/gCOD	OLR: 2.8 gCOD/L.d (10 % v/v hydrolysate)	
Wheat straw	Batch, 118 mL (40 mL working volume), 55 °C	HWE in 3 steps: a. 80˚C, 6 min b. 180 ˚C, 15 min c. 190 ˚C, 3 min	60 days	0.384 ± 0.08 m^3 CH_4/kg VS	OL: 6.3 gCOD/L (50 % v/v hydrolysate)	Kaparaju et al. (2009b)
Sorghum sundanense	Batch, AMPTS, 500 mL, 35 ± 0.5 °C	40˚C, 1 hour	31 days	0.242 m^3 CH_4/kg VS	S/I: 1 g VS/ g VS	Sambusiti et al. (2013)
		100˚C, 1 hour		0.282 m^3 CH_4/kg VS		
		160˚C, 1 hour		0.273 m^3 CH_4/kg VS		
Wheat straw		40˚C, 1 hour		0.201 m^3 CH_4/kg VS		
		100˚C, 1 hour		0.199 m^3 CH_4/kg VS		
		160˚C, 1 hour		0.224 m^3 CH_4/kg VS		
Wheat straw	Batch, 1130 mL (1000 mL working volume), 37 °C	200˚C, 10 min	60 days	0.206 m^3 CH_4/kg VS	S/I: 1	Chandra et al. (2012b)

Rice straw	Batch, 1130 mL (1000 mL working volume), 37 °C	200˚C, 10 min	60 days	0.133 m^3 CH$_4$/kg VS	S/I: 1	Chandra et al. (2012a)
Rice straw	Batch, 1000 mL (800 mL working volume), 35 ± 1 °C	90˚C, 15 min 150˚C, 15 min 180˚C, 15 min 210˚C, 15 min	50 days	168.9 mL$_{CH4}$/g TS 163.9 mL$_{CH4}$/g TS 175.7 mL$_{CH4}$/g TS 108.1 mL$_{CH4}$/g TS	OL: 35 g TS/L	Wang et al. (2018)
Oil palm empty fruit bunches (EFB)	Batch, 320 mL, 55 °C	230˚C, 15 min	45 days	0.208 m^3 CH$_4$/kg VS	OL: 39 g VS/L	O-Thong et al. (2012)
Giant reed (*Arundo donax*)	Batch, 323 ml (100 mL working volume), 53 °C	150˚C, 10 min 150˚C, 20 min 180˚C, 10 min 180˚C, 20 min	39 days	0.301±0.0064 m^3 CH$_4$/kg VS 0.293±0.0044 m^3 CH$_4$/kg VS 0.337±0.0086 m^3 CH$_4$/kg VS 0.283±0.0040 m^3 CH$_4$/kg VS	OL: 4 g VS/L	Di Girolamo et al. (2013)
Sunflower oil cake	Batch, 300 mL, 35 ± 1 °C	25 to 200˚C at 25˚C interval for 1,2,4 and 6 h	8 days	310 ± 4 mL CH$_4$/gCOD	S/I=0.5 (VS/COD basis)	Fernandez-Cegri et al. (2012)
Napier grass	Batch, 250 mL (160 mL working volume), 35 ± 1 °C	175°C for 15 mins	42 days	33.9±1.0 NmL CH$_4$/g sCOD	NA	Phuttaro et al. (2019)
Safflower stalk	Batch, 118 mL, 37 °C	180°C for 1 hour	45 days	406.9 mL/g VS	I/S=2 (VS mass ratio)	Hashemi et al. (2019)
Norway spruce (*Picea abies*)	Batch, 100 mL syringes, 55 °C	140 °C for 300 min 170 °C for 90 min	38 days	290 NmL/gCOD 195 NmL/gCOD	OL: 6 gCOD/L	(Ghimire et al., 2020)

NA: Not Available; OL: Organic Loading; OLR: Organic Loading Rate; F/M: Food/Microorganism; S/I: Substrate/Inoculum

5.2 Steam pretreatment

Steam pretreatment has also been considered to enhance methane production primarily from agricultural residues (Table 4). Increased methane yields (20-130 %) are observed at different pretreatment temperatures (120-190°C) and residence times (10-60 min)**.** L-AD of hydrolysate of woody biomass after steam pretreatment is still lacking, and only SS-AD of birch, a woody biomass, after steam pretreatment has been examined (Vivekanand et al., 2013).

Table 4: Methane production from hemicellulose from steam pretreatment of different lignocellulosic biomass.

Lignocellulosic biomass	Reactor type, mode and volume	Applied pretreatment	Digestion period	Methane yield	Loading rate	Reference
Coffee husk	Batch reactor, 160 mL (100 mL working volume), 35˚C	Steam explosion a. 120˚C, 60 min b. 180˚C, 15 min	27 days	0.137±0.0027 m^3 CH$_4$/kg VS 0.123±0.0022 m^3 CH$_4$/kg VS	S/I: 0.5 g VS/g VS	Passos et al. (2018)
Coffee husk	Batch, 160 mL (80 mL working volume), 35±0.5˚C	Steam explosion a. 120˚C, 60 min b. 180˚C, 15 min	25 days	0.145 NmL CH$_4$/g COD 0.128 NmL CH$_4$/g COD	F/M: 0.7 g COD/g VS$_{inoculum}$	Baêta et al. (2017)
Wheat straw	Batch, 250 mL, 37.5˚C	Steam explosion 180 ˚C, 15 min	NA	0.331 m^3 CH$_4$/kg VS	S/I: 1:3	Bauer et al. (2009)
Late harvested hay	Batch, 250 mL, 37.5˚C	Steam explosion 175°C, 10 min	NA	0.281 m^3 CH$_4$/kg VS	S/I: 1:3	Bauer et al. (2014)
Oat straw	Batch, 1.12 L, 37˚C	Steam explosion 190˚C, 10 min	98 days	0.201 m^3 CH$_4$/kg VS	OL: 4.3 g VS/L	Dererie et al. (2011)
Rice straw	Batch, 37.5°C, 118 mL	Steam explosion, 160-205°C, 15 min	60 days	486 NmL/gVS	I/S: 2 gVS/gVS	Aski et al. (2018)
Wheat straw Corn straw Sugarcane bagasse	Batch, 0.5 L (0.36 L working volume), 37 °C.	Steam explosion, 150 °C, 40 mins	12.5 days	195±1 mL CH$_4$/gCOD 369±22 mL CH$_4$/gCOD 178±11 mL CH$_4$/gCOD	I/S: 2 gVS/gVS	(Buitrón et al., 2019)

Agave bagasse				230±8 mL CH_4/gCOD		
Agave bagasse	Batch, 125 mL, 36 °C	Steam explosion, 0.38 MPa	30 days	317 mL CH_4/gCOD	I/S: 2 gVS/gVS	(Weber et al., 2019)

NA: Not Available; OL: Organic Loading; VS: Volatile solid; F/M: Food/Microorganism; S/I: Substrate/Inoculum

5.3 Pyrolysis

Pyrolysis is integrated with AD in order to improve the overall carbon and energy utilization efficiency (Corton et al., 2016). The integration is divided into three main types, anaerobic digestion-pyrolysis (AD-Py), pyrolysis-anaerobic digestion (Py-AD) and anaerobic digestion-pyrolysis-anaerobic digestion (AD-Py-AD). Out of these, Py-AD and AD-Py-AD are of main interest to convert lignocellulosic biomass into liquefied bio-oil (APL) for AD, while AD-Py may increase the net energy gain (Monlau et al., 2016). AD-Py integration is not relevant in this review as the end process is not AD, and readers interested in such integration are referred to the review by Feng and Lin (2017).

The combined process Py-AD utilizes the pyrolysis product APL as feedstock for AD (Torri et al., 2020) (Figure 4). The other pyrolysis products like non-condensable gases and biochar can also be utilized as AD feeds. Non-condensable gases can be fermented and/or converted to methane in AD (Pecchi & Baratieri, 2019). Some of the biochar can be used as an additive in AD to help stabilize the process by adsorption of inhibitors, enhance buffering, binding and acclimatization of microbial cells (Masebinu et al., 2019), resulting in increased methane yield (Torri & Fabbri, 2014). Application of biochar and anaerobic fermentation of non-condensable gases in AD is beyond the scope of this paper and the reader is referred to the review by Masebinu et al. (2019) for a survey of these possibilities.

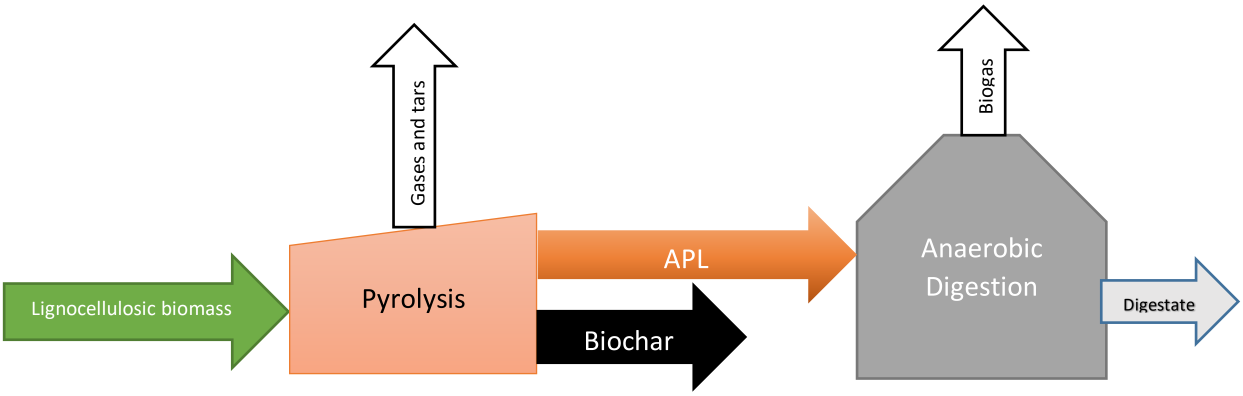


Figure 4: The flow diagram of Py-AD integration.

Pyrolysis in conjunction with AD of APL (Py-AD) is gaining interest as a low cost environmental friendly option with some investigations already started (Cordella et al., 2012; Hubner & Mumme, 2015). Life cycle analysis assessment performed on the coupling of AD has shown significant achievable reduction of greenhouse emission (Righi et al., 2016). AD of APL has been tested both through co-digestion and as a sole substrate. Inhibition is targeted in batch tests, with observed efficient anaerobic degradation of APL up to 1 g L^{-1} and complete inhibition at 20 g L^{-1} initial APL content (Willner et al., 2004a) suggesting dilution through co-digestion as a reasonable solution. Co-digestion of up to 6.5 % (v/v) pyrolignitic acids (similar to APL), from pyrolysis of wood residue, mixed with swine manure is reported for a biofilm based AD (Andreoni, 1990). Recently, APL has been used as an additive during the AD of swine manure and it is observed improved methanogenic capacity because of trace elements in APL and enhanced resistance of microorganisms to high ammonia nitrogen (TAN) concentration and its accumulation (Yu et al., 2020).

Recently, APL from woody biomass has been subjected to AD for methane production. APL from Douglas Fir wood has been examined in AD and increased methane production was observed in acetic acid (10 %) washed biomass compared to untreated, due to increased concentration of levoglucosan and reduced concentration of hydroxyacetaldehyde (Liaw et al., 2020). Continuous AD of APL from pine wood in UASB showed that 52 % of APL was potentially biodegradable at organic loading rate of 1.5 gCOD L^{-1} day^{-1} (Torri et al., 2020). Diluted APL has also been used as an additive during AD of swine manure resulting in improved methanogenic capacity because of enhanced resistance of microorganisms to high ammonia nitrogen (TAN) concentration (Yu et al., 2020). This should trigger more research on adaptation to this challenging substrate in continuous flow AD to make it feasible for industrial scale. Optimized integration of pre- and post-treatments together with adapted mixed AD cultures may be the key for full-scale AD of APL (Fabbri & Torri, 2016).

The AD-Py-AD combined process (Figure 5) is applied to handle sludge from wastewater treatment, where the AD stabilizes the sludge, makes it easier to dewater and reduces the amounts by converting a significant fraction to biogas (Tchobanoglus et al., 2003). Using pyrolysis to handle AD digestate rich in lignocellulosic substances can be a sustainable solution to further reduce sludge volumes and convert more sludge to biogas. Currently, digestate is mainly used as soil amendment, dumped in landfills or burnt at the cost of energy, which are not sustainable due to a range of negative environmental aspects (Fabbri & Torri, 2016).

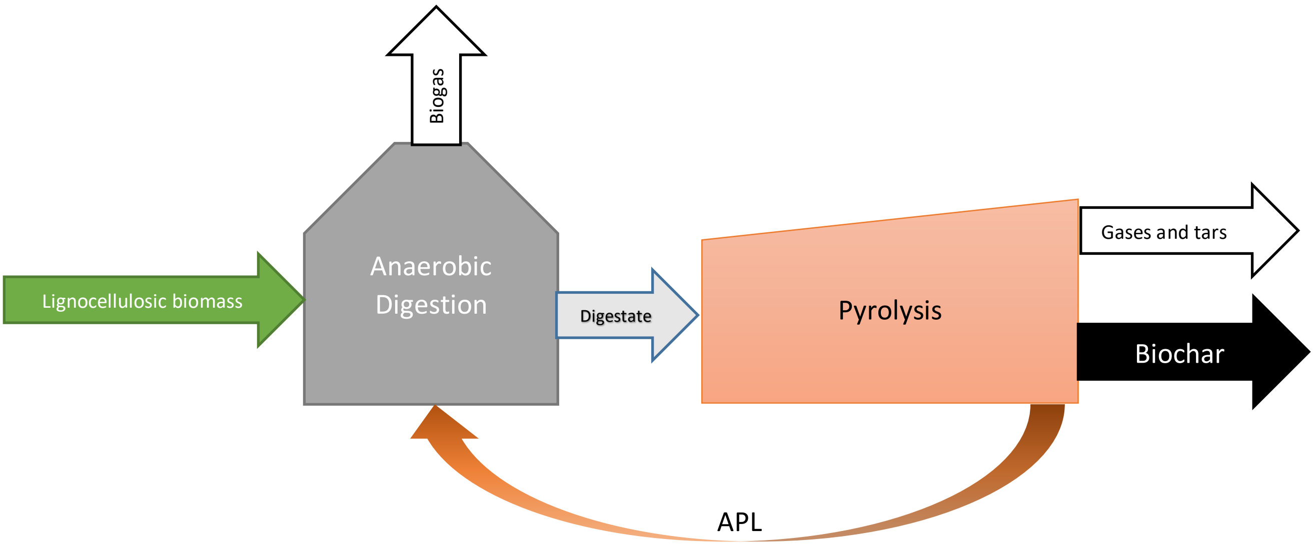


Figure 5: The flow diagram of AD-Py-AD integration.

In the AD-Py-AD process about half of volatile matter in sludge digestate can be converted to bio-oil which can reach energy content values comparable to diesel (Cao & Pawłowski, 2012). Utilizing AD-Py-AD, this energy or parts of it can be converted to biogas by AD (Seyedi et al., 2020a).

Manure is also targeted by AD-Py-AD with promising results. AD of APL from pyrolysis of AD digestate of cow manure and maize (4:3 ratio) gave a COD removal of 63 % and methane yield of 220 mL/g COD without any additives at a COD load of 12 gCOD/L, while permanent inhibition was observed at a COD load of 30 g/L (Hubner & Mumme, 2015).

Table 5: Methane production utilizing APL from various lignocellulosic biomass.

Lignocellulosic biomass	Reactor type, mode and volume	Applied pretreatment	Digestion period	Methane yield	Loading	References
Pine wood	Batch, 700 mL, 37˚C	Fluidized bed flash pyrolysis at 460˚C for 1 sec	28 d	480 NmL CH_4/g substrate	1 g APL / 700 mL	Willner et al. (2004b)
Corn stalk	Batch, 100 mL syringe (30 mL effective volume), 40 ˚C	Fixed bed intermediate pyrolysis at 400 ˚C for 10 min	225 d	126 mL CH_4/g bio-oil	OL: 35 g COD/L F/M: 0.6	Torri and Fabbri (2014)
Digestate of cow manure and maize (4:3)	Batch, 100 mL syringe (20 mL effective volume), 40.5±1 °C	Pyrolysis at 300 ˚C, 400 ˚C and 500 ˚C for 45±15 min	49 d	199.1±18.5 mL/g COD	OL: 0.129 to 49.1 g COD/L	Hubner and Mumme (2015)
Pine wood (softwood)	Continuous (UASB) reactor, 80 mL, 40 °C	Pyrolysis at 400 °C for 30 min	79 d	34 % of fed COD	OLR: 1.25 gCOD/L.d	Torri et al. (2020)

NA: Not Available; OL: Organic Load; OLR: Organic Loading Rate; F/M: Food/Microorganism; UASB: Up-flow Anaerobic Sludge Blanket; COD: Chemical Oxygen Demand

6 Pretreatment inhibitors during AD

The most prominent AD inhibitors produced during hydrothermal pretreatment are furfural and HMF together with soluble lignin and its derivatives. A range of measures has been suggested to counteract inhibition problems (Jonsson & Martin, 2016; Kim, 2018). Readers interested in details regarding detoxification of hydrolysates are suggested review articles by Bhatia et al. (2020) and Kumar et al. (2019).

The pyrolysis process also produces several toxic compounds to microorganism at percentage level but several studies have shown that AD inocula adapt to these compounds (Barakat et al., 2012; Benjamin et al., 1984). The ratio of inhibiting compounds to inoculum influences the adaptation process (Park et al., 2012). Powdered activated carbon (PAC) and biochar have also been found useful in mitigating inhibitory effects (Liu et al., 2017; Torri & Fabbri, 2014). Although biochar cannot be degraded to produce methane (Mumme et al., 2014), it can be added during AD to help in detoxification of APL while also supporting biofilm, favoring biomethanation or enhancing electron transfer thereby enhancing the biogas production (Masebinu et al., 2019). Developing a robust microbial consortia tolerant to toxicity of APL through metabolic evolution of organism has also been found effective for successful AD (Zhou et al., 2019).

6.1 Sugars derivatives

Sugar can degrade to furanic compounds which at high concentrations hamper microorganisms by inhibiting cell growth, inducing DNA damage and inhibiting several enzymes of the glycolysis pathway (Palmqvist & Hahn-Haagerdal, 2000). The concentrations observed for partly or complete inhibition on methanogenic activity from HMF and furfural is in the range 2-4 g/L for furfural and 2-10 g/L for HMF depending on substrate compositions and inoculum in batch (Ghasimi et al., 2016). Furfural and HMF may have additive inhibitory effects when both are present (Taherzadeh et al., 1999), while furfural is found to be more inhibitory compared to HMF due to its lower molecular weight which eases its uptake by microbial cells (Quéméneur et al., 2012).

Furfural and HMF concentrations of 1 g/L each, tested separately, have been found to give no inhibition effect during mesophilic AD of xylose (1 g/L) and these compounds could be used as sole carbon sources (at ~2 g/L) to produce methane (Barakat et al., 2012). During AD both furfural and HMF can be converted to less inhibitory compounds such as furfuryl and HMF alcohols by facultative anaerobes in AD, while furfural also can be converted to furoic acid and acetate, before being converted to methane and carbon dioxide (Monlau et al., 2014). For detailed mechanism of inhibitory products formation during hydrothermal pretreatment, readers are referred to Nitsos et al. (2013).

6.2 Soluble lignin

Soluble lignin dissolved along with the hemicellulose hinders efficient AD of hydrolysate by inhibiting the growth of methanogens (Baeta et al., 2016a; Barakat et al., 2012), with negative linear correlation between lignin content and methane yield during AD of cellulose, hemicellulose, manure wastes and acetate-rich wastewater (Li et al., 2018). A reduced anaerobic digestion rate for methanogenesis, acidogenesis and hydrolysis by 15 %, 10 % and 35 % respectively at soluble lignin concentration higher than 5 g/L is observed (Koyama et al., 2017).

Negligible amounts of lignin is broken down during AD with only 2-7 % of methane produced from lignin during co-digestion of natural lignins (organosolv, kraft and lignosulfonates) with xylose (Barakat et al., 2012), and only 1.4 % biodegradation of hardwood lignin compared to 16.9 % of grass lignin (Benner et al., 1984). However, elevated AD temperature was found to enhance conversion rate of lignin and lignified substances to methane or lower molecular-weight aromatic compounds during AD (Benner & Hodson, 1985).

6.3 Lignin derivatives

Lignin derivatives such as soluble phenolic compounds are formed at pretreatment conditions above 160°C (Hendriks & Zeeman, 2009). The produced phenolic compounds have in many cases inhibitory and toxic effects on bacteria and methanogens/archaea (Campos et al., 2009) as they can damage cell membranes, causing leakage of intracellular components and lead to inactivation of essential enzymatic systems (Heipieper et al., 1994). Inhibition by lignin derived phenolic compounds is directly related to molecular weight; low molecular weight are more toxic than high molecular weight phenolic compounds (Clark & Mackie, 1984).

Microorganisms are however capable of adapting to soluble phenolic compounds (Hendriks & Zeeman, 2009) with effective phenol degradation during AD (Fang et al., 2004) and Benzoate as a key intermediate (Fang et al., 2004). AD efficiency of different phenols depends on temperature with higher degradation efficiency at mesophilic than at thermophilic conditions (Leven et al., 2012). Some degradation can also occur during the pretreatment with formation of the AD inhibiting phenolic degradation products such as syringaldehyde and vanillin (Barakat et al., 2012).

6.4 Inhibitory and toxic compounds from pyrolysis

APL contains several toxic compounds and AD containing mixed anaerobic consortia possible of adaptation to a wide range of chemical substances (Appels et al., 2011) can be exploited for its capacity to degrade and convert such to methane (Torri & Fabbri, 2014; Wen et al., 2020).

Si et al. (2018) observed complete conversion of furfural and HMF to methane while some phenolic compounds such as 4-ethyl-phenol and 3-hydroxypyridine degraded with some inhibition in acetogenesis during continuous AD.

Pyridine and pyridine derivatives can also be converted by AD at low biodegradation rates (Li et al., 2001). Sun et al. (2011) observed a synergistic effect of the inhibitors found in APL, in which phenol > 400 mg/L inhibited pyridine degradation. However, inhibition caused by phenol on pyridine degradation was less in immobilized compared to suspended cultures (Kim et al., 2006) implying that microbial aggregates (e.g. biofilm and granules) may handle APL toxins better than reported batch tests.

7 Pretreatment tuning to increase overall and AD yield

Pretreatment conditions should be chosen based on primary objective while also considering the utilization of by-product streams for enhanced resource recovery. The whole process should be optimized according to the total set of products, including methane production by AD of the liquid. In this perspective, efficient pretreatment must extract hemicellulose while minimizing carbohydrates loss and limiting inhibitors formation in the total process (Benjamin et al., 1984) so that the hydrothermal extraction can produce value-added products, such as biogas, from compounds that are otherwise wasted in other pretreatment schemes (Liu, 2015).

7.1 Hydrothermal- Effect of temperature

Biomethane potential of hydrolysate cannot be predicted on the basis of hydrothermal treatment alone due to factors such as difference in hemicellulose content between different lignocellulosic biomass, generation of inhibitors, hydrothermal reactor configuration, liquid and solid ratio, operation mode i.e. batch or continuous and possible utilization of catalytic agent (Ahmad et al., 2018).

The biomethane potential is however clearly influenced by hydrothermal pretreatment temperature with some optimal combination of time and temperature determining the amount of sugars transferred to the liquid hydrolysate and the amounts of inhibitory compounds included. The choice of pretreatment severity (based on temperature and residence time) depends on the feedstock. Low severity pretreatment conditions form products such as oligosaccharides (xylooligosaccharides) of higher potential value due to their prospective use in medicinal, food, cosmetic and health products (Qing et al., 2013). Higher severity leads to better extraction of hemicellulose from the biomass but forms inhibitors from degradation of monosaccharides extracted, with possible appearance of metal ions from reactor vessel in the extracted hydrolysate (Carvalheiro et al., 2016).

Recent studies suggest that operating temperature between 100 and 230 °C is best to obtain hydrolysate for biogas production (He et al., 2015) and hydrothermal pretreatment temperatures above 250˚C is not recommended to avoid unwanted pyrolysis reactions (Dahadha et al., 2017). If biochar is the main product of the process, temperature should be chosen (dependent on the biomass type) to extract hemicellulose as much as possible while avoiding lignin and cellulose solubilization. Review by He et al. (2015) covers specific operational parameters crucial for lignocellulosic biomass for biogas production. These aspects are beyond the scope of this review.

7.1.1 Hot water extraction

The hydrolysate composition after hot water extraction vary depending upon the origin of lignocellulosic biomass and process conditions such as reaction temperature, solid to liquid ratio, type of reaction vessel and mode of operation, i.e, batch or continuous (Ahmad et al., 2018).

Temperature affects the extraction mass removal (Lu et al., 2012), where an optimal extraction severity can be observed. E.g. HWE of *sorghum sundanense* treated at 100°C for 1 hour produced the highest yield (0.282 m^3 CH_4/kg VS) of the conditions tested and treatment at lower and higher temperatures led to lower AD methane yield (Sambusiti et al., 2013).

A higher extraction pressure maintains the liquid hot water as solvent to solubilize mainly the hemicellulose, which makes the cellulose better accessible and reduces the formation of inhibitors (Hendriks & Zeeman, 2009; Liu, 2015). pH can also influence the formation of inhibitors and should be maintained between 4 and 7 (Mosier et al., 2005a). This optimized pH also maximizes the solubilization of the hemicellulose fraction and reduces the formation of monomeric sugars and therefore also the formation of inhibitory degradation products (Mosier et al., 2005a).

7.1.2 Steam pretreatment

Optimal steam pretreatment/steam explosion treatment depending on the characteristics desired for the product can be achieved by manipulating parameters like temperature, residence time, catalyst dosage, time of pre-soaking, particle size and moisture content of the substrate (Talebnia et al., 2010). However, clear correlation between different parameters are not yet found (Simangunsong et al., 2018). As in HWE, higher temperature and retention time leads to higher extraction of hemicellulosic sugars but also the inhibitor concentration increased due to degradation of sugars.

Although the effect of particle size of the biomass in pretreatment is not much discussed, reducing the particle size before pretreatment is crucial for the optimization of sugar conversion as particle size has a major influence on the kinetics of the hydrolytic process (Ballesteros et al., 2000). Small particle leads to easy and high extraction of hemicellulosic sugars, whereas the largest particle may lead to lower extraction (Simangunsong et al., 2018). However, concentration of inhibitors (furfural and HMF) is higher during the steam pretreatment of smaller particles (Cullis et al., 2004). Maximum hemicellulose extraction with low concentration of inhibitors and soluble lignin should be in focus when the hydrolysate is meant for AD. Lignin and cellulose is preferred in the solid residue used in pyrolysis for biochar and bio-oil production.

7.2 Pyrolysis

Pyrolysis parameters should be tuned for higher APL yield if the side stream is to be treated by AD, producing bio-oil. This will increase easily degradable ketones and acids and decrease the concentration of recalcitrant or toxic carbon compounds such as hydroxyacetaldehyde, acetol, furans, N-heterocyclic compounds and phenols in APL for enhanced methane yield (Alvarez et al., 2014; Rezaei et al., 2014). Parameter tuning should be chosen accordingly if biochar or syngas production is also considered, where lower process temperature and longer vapor residence time favors char production and high temperature and longer residence time increases syngas formation (Bridgwater, 2012).

Physical, thermal, chemical and biological pretreatments on biomass before pyrolysis can also help in avoiding inhibitors and increasing sugar concentration in the APL, to favor AD methane production. HWE before pyrolysis removes hemicellulose and alkali metals (Na and K) leading to increased sugar concentration, mainly levoglucosan, while reducing possible AD inhibitors such as acetic acid, carboxylic acids, ketones and phenols in the bio-oil (Chang et al., 2013; Kan et al., 2016). Similarly, steam explosion pretreatment also reduces acids, furfural and HMF concentration in the bio-oil while increasing the concentration of levoglucosan and other anhydrous (Hassan el et al., 2009; Kan et al., 2016) increasing the methane production (Liaw et al., 2020). Comprehensive reviews on details of such pre-treatments are published by Alvarez-Chavez et al. (2019) and Kumar et al. (2020).

8 Concluding remarks

Hydrolysate from hot water extraction and steam pretreatment is a suitable AD feed due to high organic carbon content and low concentration of inhibitors. However, a lower pretreatment severity is favorable to use hydrolysate as AD feed. APL produced from pyrolysis is a complex feed for AD due to the presence of various known and unknown complex inhibitors but evidence for its feasibility is found in the literature. Pre-treatment of biomass before pyrolysis, adaptation of microorganism to inhibitors and additives such as biochar can help the AD cultures cope with inhibitors in APL.

Nomenclature

5-HMF: Hydroxymethylfurfural

AD: Anaerobic digestion

AFEX: Ammonia fibre explosion

APL: Aqueous pyrolysis liquor

C/N: Carbon to nitrogen ratio

COD: Chemical oxygen demand

CSTR: Continuous stirred-tank reactor

DNA: Deoxyribonucleic acid

HTP: Hydrothermal pretreatment

HWE: Hot water extraction

L-AD: Liquid-state anaerobic digestion

LCA: Life cycle assessment

LHW: Liquid hot water

PAC: Powdered activated carbon

PBR: Packed bed reactor

PHA: Polyhydroxyalkanoate

SS-AD: Solid-state anaerobic digestion

TAN: Total ammonia nitrogen

TS: Total solids

UASB: Up-flow anaerobic sludge bed

VFA: Volatile fatty acid
Py: Pyrolysis

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