

Investigation on the Use of Intermediate Crops for Anaerobic Digestion as a Renewable Source of Energy



LUND
UNIVERSITY

Ida Ahlberg and Thais Nilsson

Department of Chemical Engineering
Master Thesis 2015

Investigation on the Use of Intermediate Crops for Anaerobic Digestion as a Renewable Source of Energy

by

Ida Ahlberg and Thais Leidiomara Silva Nilsson

Department of Chemical Engineering

Lund University

June 2015

Supervisor: **Associate Professor Ola Wallberg**
Co-supervisor: **Assistant Lecturer Emma Kreuger**
Examiner: **Associate Professor Mats Galbe**

Picture on front page: Intermediate crops at Kronoslätt Farm. Photo by Jeppa Olanders.

Postal address

P.O. Box 124
SE-221 00 Lund, Sweden

Web address

www.chemeng.lth.se

Visiting address

Getingevägen 60

Telephone

+46 46-222 82 85

+46 46-222 00 00

Telefax

+46 46-222 45 26

Preface

This master thesis has been performed at Chemical Engineering at the Department of Chemical Engineering at Lund University at the Faculty of Engineering, LTH, Lund University.

For this master thesis project Ola Wallberg from the Department of Chemical Engineering and Emma Kreuger from the Department of Biotechnology were our supervisors. We would like to thank our supervisors for providing us with advice, all the necessary information and for their regular feedback. We would also like to take the opportunity to acknowledge our examiner Mats Galbe for his time and effort in this project. This project was included in the Metanova project financed by Energimyndigheten (31090-2).

We want to thank Filip Vrgoc at Department of Chemical Engineering at Lund University who patiently assisted us in a great part of the laboratory work. Also we are grateful towards Jeppa Olanders for letting us harvest the intermediate crops to our experiments at Kronoslätt Farm and Sven-Erik Svensson at Swedish University of Agricultural Sciences for the help and advice about the intermediate crops, Balazs Franko and Pia-Maria Bondesson at Department of Chemical Engineering at Lund University, for all the help and knowledge they have provided us with issues with the laboratory experiments. We would also like to thank Victor Gustafson, our lab colleague who made our days much better during this master thesis.

The intermediate crops used in this thesis were grown at Kronoslätt Farm in the project called “Intermediate Crops after Whole Crop Silage as Biogas Substrate” and financed by Region Skåne, Partnerskap Alnarp (PA 790) and Skånska Biobränslebolaget (SB3).

Finally we would like to thank our families and friends for supporting us throughout the work with this project.

Ida Ahlberg and Thais Leidiomara Silva Nilsson

Lund, June 2015

Abstract

The eminent dilemma around the relationship between the increasing world population, the need of new fuel sources and environment issues has raised great awareness in the last decades. The European Parliament has set common goals such as the reduction of the greenhouse gases by 20 % by the year of 2020, followed by the requirement of at least 10 % of the fuel has to be biofuel. An adjacent issue is the source of the biofuel, which are currently most of agricultural crop nature. However these energy crops should not compete with food crops and favourably have other features that promotes its usage. Intermediate crops are for example a promising resource since it may reduce the risk of the nutrients leaching since the crop can take up nutrients in the soil.

The aim of this thesis was to investigate the potential of six different intermediate crops (hemp, oilseed radish, white mustard, phacelia, sudangrass and hairy vetch) as energy resources under anaerobic digestion condition after 30 days. Hairy vetch showed the highest methane yield (343 m³/ t VS) followed by sudangrass (316 m³/ t VS). Sudangrass showed a slight potential to increase the methane yield (97 % of theoretical yield based on the component analysis for the sudangrass) if steam pretreatment is for instance applied. This relatively high yield result could be attributed to the relatively low lignin content of sudangrass compared to other crops (16 % of TS). An important factor taken into consideration when selecting which crop to investigate for pretreatment was also the methane yield per hectare for sudangrass which had the second highest value (995 Nm³/ ha) after oilseed radish (1217 Nm³/ ha). Other factors were also taken into account in the decision such as: total solids content, how easily the crop is managed in agriculture for example due to water content and the negative impact of shared diseases with other crops.

Based on the results and factors discussed above, the crop which had the greatest potential for methane yield improvement but also availability of ensiled material, sudangrass, was further investigated for the pretreatment effects on methane production. The ensiled sudangrass was pressed into a liquid fraction and a solid fraction, where the latter was taken forward to the pretreatment step. The pretreatment conditions studied were steam pretreatment with added catalyst (1 % acetic acid or 2 % sodium hydroxide weight percentage base on total solid of sudangrass, sprayed in the crop) and it was compared to steam pretreatment alone. Also different temperatures (180 °C, 190 °C, 200 °C and 210 °C) and retention times (5 and 10 min) were studied. The sodium hydroxide impregnated crop did not show better yield than the ensiled sudangrass. Quite the contrary: the alkaline catalyst showed in the best case scenario (190 °C and 10 min) a methane production decrease of 12 % compared to the solid fraction of ensiled sudangrass (325 m³/ t VS). On the other hand the acid treatment at 190 °C and 5 min residence time showed an 11 % increase in the same context. The acid treatment at 190 °C and 5 min showed the highest final methane yield (362 m³/ t VS).

Conclusively it could be said that all the intermediate crops have a potential for usage in methane production, assuming other aspects are optimized for its usage. Pretreatment can be with advantage be used to improve the methane yield, where there is room for improvement. The question is rather what specific combination of pretreatment conditions will yield the best enhancement and careful investigations should be made before determining the ultimate pretreatment for a specific crop.

Populärvetenskaplig sammanfattning

- **En undersökning av biogaspotential för färska mellangrödor och ångförbehandlad ensilerat sudangräs.**

I detta examensarbete har sex olika mellangrödor (hampa, honungsört, oljerättika, sudangräs, luddvicker och vitsenap) undersökts för användning till biogas produktion som ett alternativ för framställning av bibränsle.

Miljöfrågor, behovet av nya bränslen och ökningen av världens befolkning har lett till uppmärksamheten för förnybara bränslen det senaste årtiondet. Den Europeiska Unionen har satt gemensamma mål att minska utsläppen av växthusgaserna med 20 % till år 2020, vilket följs av att minst 10 % av bränslena i transportsektorn ska vara förnybara. Biogas är ett av de befintliga alternativa bränslena. Dessutom är det mycket fördelaktigt om råvaran för biogasen inte konkurrerar med jordbruksareal avsedd för livsmedel. De så kallade mellangrödorna blir i kontexten ett mycket bra alternativ eftersom de förebygger kväveläckage genom att fånga upp näringsämne från jorden.

En viktig fråga är källan till biobränslet, vilket för närvarande är mest jordbruksgrödor, såsom majs och vete. Den energigröda som används till produktion av biobränsle bör dock inte konkurrera med livsmedelsgrödor utan även ha andra funktioner som främjar dess användning. Mellangrödor är ett lovande material då de bland annat kan förbättra kvaliteten i jorden genom att binda näringsämnen och minska näringsläckaget till närliggande vattendrag. Mellangrödor etableras och skördas i ett intervall i grödsekvensen eller växtföljden då marken ligger oanvänd för annan odling, mellan två huvudgrödor. Huvudgrödorna kan till exempel vara potatis, höstvete eller raps.

Biogas består till största delen av metan och därefter koldioxid. När våra undersökningar gjordes på metanutbyte blev det oljerättika som gav bäst resultat per hektar, följt av sudangräs. För sudangräs ansågs det finnas utrymme för förbättringar med hjälp av förbehandling. Ensilerat sudangräs valdes därför för ytterligare undersökning. Detta utifrån bland annat beslut om att ensileringen av material blivit bra, hur lätt materialet är att hantera i jordbruket och resultat för metanutbyte per hektar. Undersökningen delades upp i två typer av förbehandlingar, en där det ensilerade sudangräset impregnerades med en syra och en där det impregnerades med en bas. Det ensilerade sudangräset sparades i en vätskefas och en fast fas. Den fasta delen av sudangräset behandlades med ättiksyra eller med natriumhydroxid. Därefter ångförbehandlades sudangräset och under ångförbehandlingen testades olika temperaturer och uppehållstider. Den förbehandling som gav högst metanutbyte var syra-katalyserad sudangräs ångförbehandlat i 190 °C och med 5 minuters uppehållstid. Undersökningen visade att sudangräs katalyserad med natriumhydroxid som bas inte gav bra resultat, då metanutbytet var lägre än både färskt material och ensilerat sudangräs.

Analysen på såväl färska mellangrödor, ensilerat material samt förbehandlat sudangräs har gjorts med avseende på komponentanalyser och undersökningar för metanutbyte. Det har tagits fram hur mycket socker, biprodukter och lignin som de olika materialen innehåller. Med analys av dessa resultat tillsammans med resultat för metanutbyte gjordes slutsatser både för färska mellangrödor och för det förbehandlade sudangräset. Slutligen kom vi fram till att syrakatalyserad ångförbehandling på sudangräs gav bättre metanutbyte än baskatalyserad.

Contributions to the paper

Ida and Thais contributed to all the experimental work, the analysis of the results and in the writing of the manuscript.

Ida – performed the analysis of the results from steam pretreatment with acetic acid as catalyst.

Thais – performed the analysis of the results from steam pretreatment with sodium hydroxide as catalyst.

Abbreviation

AD	anaerobic digestion
AIL	acid insoluble lignin
AMPTS	automatic methane potential test system
ASL	acid soluble lignin
DM	dry matter (equal to TS in this master thesis)
ES	ensiled sudangrass
FS	fresh sudangrass
GC	gas chromatography
HAc	acetic acid
HMF	hydroxymethylfurfural
HPLC	high-performance liquid chromatography
IBC	intermediate bulk container
IC	intermediate crops
LCFA	long chain fatty acids
LF	liquid fraction
NaOH	sodium hydroxide
SF	solid fraction
SP	steam pretreatment
VFA	volatile fatty acids
WIS	water insoluble solids
SE	steam pretreated

Table of contents

1	Introduction.....	1
1.1	Aim	1
2	Background	2
2.1	Biofuels today	2
2.2	Biogas	2
2.3	Intermediate crops.....	3
2.4	Different parts of the plant: cellulose, hemicellulose and lignin.	4
2.5	Anaerobic digestion	4
2.6	Pretreatment.....	5
2.6.1	Different pretreatments	5
2.7	Sudangrass.....	7
3	Material and methods.....	9
3.1	Raw material	9
3.2	Steam pretreatment	9
3.3	BMP analysis.....	10
3.4	Analysis	10
3.4.1	Determination of total solid content and volatile solid content	10
3.4.2	Determination of water insoluble solids, WIS	11
3.4.3	Composition analysis	11
3.4.4	Determination of sugar content	11
3.4.5	Determination of protein content in the intermediate crops.....	12
3.4.6	Correction of TS and VS content for ensiled materials	12
3.4.7	Assumed methane content in the AMPTS flasks	13
3.4.8	Extraction on pretreated sudangrass.....	13
3.5	Severity factor	13

4	Results and discussion	15
4.1	Intermediate crops.....	15
4.1.1	Raw material compositions	15
4.1.2	Methane potential/Anaerobic digestion	17
4.2	Steam pretreatment	21
4.2.1	Acetic acid.....	22
4.2.2	Sodium hydroxide	28
4.2.3	Combined results.....	33
5	Conclusions.....	37
6	Future work.....	38
7	Reference list	39

Appendices

1 Introduction

Due to increasing concern upon the effect of, e.g. greenhouse gases and crude oil price, biogas has become of major interest as an alternative energy source. Intermediate crops (IC) are interesting as a resource of biomass because they may not compete with soil usage for food or feed crops. Also trends on the EU regulations points to the fact that less agricultural land will be provided for energy sequestration [1]. In order to make intermediate crops a profitable resource for biogas production, it is necessary to investigate and improve the methane outcome before utilizing the biomass in an industrial scale. Studies have shown that pretreatment can increase the enzymatic accessibility, thus increasing the hydrolysis degree of lignocellulosic materials, for example for digestion to methane [2,3].

1.1 Aim

The aim of this master thesis was to investigate the possibility of using IC for methane production through anaerobic digestion (AD) as a renewable source of energy. This was accomplished by evaluating the biochemical methane potential (BMP) for six different IC: hemp (*Cannabis sativa*), oilseed radish (*R. sativus var. Oleiferus*), white mustard (*Sinapis alba*), phacelia (*Phacelia tanacetifolia*), sudangrass (*Sorghum sudanese*) and hairy vetch (*Vicia villosa*). Finally this project also aimed at investigating the importance of pretreatment and its effect on ensiled sudangrass (ES) by investigating which effects steam pretreatment alone or steam pretreatment coupled with acetic acid (CH_3COOH) or sodium hydroxide (NaOH) catalyst will give on the biogas yield.

2 Background

2.1 Biofuels today

The increasing concern associated with the need for fuels and chemical resources and it has become clearer than ever during the past decades that solutions should be found. As the fossil fuels reserves are limited, the society is faced with the growing demand for renewable fuels. Biofuels originated from various kinds of biomass is a vital answer to that concern [4].

The biofuels made from soluble sugars, starch or vegetable oils, found in arable crops such as corn and sugar beets, are called first generation biofuels. This materials can easily be converted using conventional technology. However those crops are often classified as food crops, which immediately conflicts with the hunger problem debates all around the globe. An alternative is the so-called second generation fuels. That sort of biomass comprises any source of organic carbon which can be renewed rapidly as a part of the carbon cycle. This includes lignocellulosic biomass, such as agricultural residues and woody crops. The major problem with the second generation biomass is that the useful sugars present in the feedstock are physically blocked by the lignin, and also how the lignocellulosic complex is bound presents difficulties [5].

Nonetheless the issue related to the difficulty in generating biofuels from the second generation biomass has lately been pushed towards finding a solution. The European Commission Renewable Energy Directive decided that by the end of 2020 the use of renewable energy should contribute with 20 % [6]. In Sweden the projections are made by the Swedish Energy Agency. The target is that the share of renewables in the transport sector will reach 10.4 % in 2020. Today the biomass contributes with 4 % of the total energy supply in Europe and 11.8 % in Sweden [6,7]. This increase of biomass is expected to come from agricultural land and forestry [1,8].

The proportion of renewable energy in Sweden was 33 % in 1990 [6], and has now increased to 51 % (in 2012) [9]. In 2020 the EU target for Sweden is set to 49 % and Sweden has an additional goal that at least 50 % of the final energy use will come from renewables, which now is fulfilled. In the transport sector, Sweden's ambition is in long-term to be independent on fossil fuels by 2030 [6].

2.2 Biogas

The second generation biofuels can be manufactured from different types of biomass and there are a couple of existing full-scale biofuel plants. For instance in São Miguel dos Campos in Brazil the first commercial-scale cellulosic ethanol plant began the production in September 2014 [10]. Biogas is yet another example on the expanding biofuel full-scale plants all over the world. Sweden's largest biogas plant, Swedish Biogas International Jordberga, produces about 11.7 million Nm³/ year bio-methane, using raw materials such as sugar beet and wheat straw, but also small amounts of intermediate crops have been tested [11].

When discussing methane production from crops, the debate is not new. In the 1930's studies on biomethanation potential of different crops were done in the USA. Investigations were done in the 1950's in Germany and 1980's in New Zealand. The digestion of the crops were demonstrated but it was not considered economically feasible. However due to higher oil prices

the crop research and development were stimulated. The 100 digesters in Germany in 1990 have increased to 6000 biogas plants in 2010 [12]. In Sweden there are 233 biogas production facilities, which produces raw biogas from the digestion of raw material [6]. Raw biogas is defined as biogas produced from digestion containing roughly 60 % methane and 29 % carbon dioxide, and some trace elements of hydrogen sulphide [13]. When using biogas as vehicle fuel, for this application it is important to have a high methane content in the gas. By removing the carbon dioxide the energy content in biogas is increased, which is in direct proportion to the methane concentration [14]. There are 47 facilities which upgrade the biogas to transport fuel quality [6,12].

Because of the new legislations in the European Union, new crops and residues have to be investigated for the production of biofuels. The new legislations aimed to reduce the agricultural land where food crops are used for biofuel production [1]. Biogas can be produced from plants that are not used directly for human food consumption. Therefore, the soil less suitable for food production can be used for the cultivation of crops [12]. By using IC some of the environmental issues may be solved and also it will increase the renewable energy production in form of biogas. This without interfering with the production of food crops [15].

2.3 Intermediate crops

The main use of the IC today is to reduce the leaching of nitrogen in the soil. IC are sown after the food and feed crops along with the rest of the mineral nitrogen in the soil. To fertilize crops, mineral nitrogen has been widely increased in the last 30 years. But due to this, the problem of nitrogen pollution in both surface and underground water have increased. The nitrogen is incorporated in the IC and will thus decrease nitrogen losses through leaching or gaseous emissions [16]. It will therefore protect the aquatic environment and the need of additional fertilizer for the next season is reduced [15,17,18]. Another alternative for IC is the so called catch crops. The catch crops are cultured to reduce the nitrogen leakage to watercourses but it will also improve the soil quality by taking up the nutrients in the soil. The catch crop is thereafter left on the farmland and in that way the upcoming crop will make usage of the nutrients, now present in the catch crops. Meanwhile the IC are cultured for mainly two purposes, for the nitrogen leakage and also for the usage as a substrate to produce biofuel [19].

A major issue today is to find a new low-cost feedstock for biogas plants with a high biogas yield to obtain a more feasible process [15]. Today most IC are ploughed into the soil, but the option is to use this harvestable IC for digestion into biogas in a biogas plant. IC can be harvested in the autumn and will then over the winter and early spring leave very low amount of nitrogen in the fields [18].

Previous studies have been done on IC, e.g. by Kreuger et al., who looked at AD of industrial hemp. The study was performed on hemp at four different harvest times between July and October in Southern of Sweden [20]. A high methane yield per hectare was shown for the hemp cultivated at southern Sweden. Another paper by Niemetz et al., investigated the economic and ecological potential assessment for biogas production based on IC [21]. On closer examination it was shown in the study that IC can play an important role in sustainable agriculture still being feasible if aspects such as social and ecological network are taking into account. Studies on the effects of IC on the sense to reduce the nitrogen fertilization on nitrogen leaching have been

done by Constantin et al. It was reported that the IC are the most efficient technique to reduce the nitrogen leaching [17].

2.4 Different parts of the plant: cellulose, hemicellulose and lignin.

The building blocks of lignocellulosic materials are mainly three components: lignin, cellulose and hemicellulose. The composition of each species varies but there are about 50 - 60 % cellulose/hemicellulose and 20 - 35 % lignin [22].

Cellulose is the most abundant macromolecule present in the plants cell-wall and because of its properties, it functions as the main structural component of the walls. Its structure can be described as a linear high-molecular-weight polymer built up of β -D glucose. In the plant, there is an organized crystalline part and an amorphous part. Those (1,4) β -D-glucan chains are mostly bonded by hydrogen bridges to one another, forming cellulose bundles [23]. Cellulose crystallinity, its association with lignin and particle surface area are factors that influences the rate of cellulose hydrolysis [3,24]. In a study by Kihlman et al., it was shown that no significant solubilisation of cellulose in NaOH/urea/thiourea (wt. % ratio 8:8:6.5) was found in steam exploded paper pulp at 210 °C but at temperature as high as 226 °C solubility was observed [25].

Hemicellulose is closely associated with the cell-wall cellulose. It consists of the three hexose-type sugars, i.e. glucose, mannose and galactose and two pentose-type sugars, i.e. xylose and arabinose. Hemicellulose has lower molecular weight than cellulose, consisting of shorter chains with side groups and sometimes branches. Those side groups could be acids, such as acetic acid, glucuronic acid, ferulic acid and *p*-coumaric acid [26,27]. Hemicellulose provides the connection between cellulose and lignin which gives rise to the rigid network that builds up the cell wall. Hemicellulose is highly water soluble. The solubility of hemicellulose is of importance for the enzymatic hydrolysis, as the extraction of the present sugars is crucial for higher material usage. The solubility will depend on temperature, pH and compositions of the biomass. The degradation of hemicellulose is influenced by the lignin [27].

In contrast to the polysaccharides cellulose and hemicellulose, lignin consists of an aromatic system composed of phenylpropane units (i.e. *p*-coumaryl, coniferyl and sinapyl alcohol). The lignin networks with the fibrils of the cell walls, providing strengthening, impermeability and resistance against oxidation and microbial attack [28]. Lignin is the most recalcitrant component of the hemicellulose [27]. Monomeric lignin can be degraded anaerobically, while oligomeric/polymeric lignin also can be degraded but the higher the degree of polymerisation the more recalcitrant it is [29].

2.5 Anaerobic digestion

The microbial degradation of organic compounds to biogas occurs under anaerobic conditions. Biogas consists mainly of methane (50 - 75 %) and carbon dioxide (25 - 50 %), and small amounts of hydrogen sulphide [30]. When fermenting a substrate in absence of air, the microorganisms in the feedstock transform the biomass waste into biogas. The solid residue can be used after the fermentation as fertilizer [30,31]. AD can contribute not only to renewable heat and electricity, but also to transport fuel [12]. The anaerobic microorganisms are quite

specialized and in order to completely degrade the many different compounds present in plants, a wide range of microorganisms are necessary [32].

The degradation process under AD can be simplified into four major steps: (1) hydrolysis, (2) acidogenesis, (3) acetogenesis and (4) methanogenesis [33]. Each step has its own microbial population and optimal conditions. The hydrolysis step is performed by extracellular enzymes that hydrolyse macromolecules into smaller molecules (e.g. carbohydrates into sugars, lipids into long chain fatty acids (LCFA) and glycerol, proteins into amino acids). In the next step, acidogenesis, sugar and amino acids are further degraded by acidogenic bacteria into volatile fatty acids (VFA) (e.g. valerate $C_4H_9COO^-$, butyrate $C_4H_7O_2^-$ and acetate CH_3COO^-), alcohols, carbon dioxide and hydrogen. In the acetogenesis VFA, LCFA and alcohols are anaerobically oxidised into acetate, carbon dioxide and hydrogen. The last step, methanogenesis, is the methane production from either degradation of acetate or by reaction of hydrogen and carbon dioxide [34].

2.6 Pretreatment

As mentioned above second generation biofuels, e.g. biogas, can be produced from lignocellulosic materials such as IC. However, lignocellulosic materials features chemical and physical barriers that will interfere in the enzymatic hydrolysis of cellulose and hemicellulose, which are the main digestible parts. Lignin will act as a physical barrier, blocking the digestible parts of the plant to the hydrolysing enzymes. Chemically, the biodegradability is often limited by the crystallinity of the glucose polymer [35]. In order to make second generation biomass competitive with starch and sugar based materials in the production of biogas, there is a clear need of some kind of treatment of the material. Despite the current technological impasses, the demand for new conscious fuel resources motivates research and development of such innovative technologies [2].

There is a large number of pretreatment technologies which have been studied [2,3,36]. Each technology has a specific impact on the different parts of the biomass: cellulose, hemicellulose and lignin. Nonetheless downstream process steps in the biofuel production should also be taken into account when choosing a desired pretreatment technology [2].

2.6.1 Different pretreatments

In order to increase the material availability to enzymatic hydrolysis for biogas formation or production of bioethanol, a pretreatment is necessary. The pretreatment should be both effective in increasing the accessibility to digestible parts but also be economically feasible. One should expose more cellulosic fibres to the hydrolysis process, but without the destruction of cellulose and hemicellulose or with limited formation of inhibitors that will retard the microorganisms responsible for the hydrolysis or fermentation. The aim is to have a low energy demand process that demands little or cheap chemicals and that produces none or harmless byproducts, but also keeping the pretreatment equipment investment as low as possible. It is clear that no pretreatment has so far succeeded to fulfil all the requirements to a satisfying degree. Different pretreatment methods and conditions have to be adapted to the material used, due to its almost unique structure and composition [22].

Several pretreatment technologies have been introduced prior to enzymatic hydrolysis or digestion. They can be classified into four major groups: physical, physico-chemical, chemical and biological [3,22]. Milling and irradiation by e.g. microwaves, gamma rays or electron beams, are examples of studied physical pretreatments. The former acts by increasing the accessible surface area and pore size for enzymatic attack [37], while the latter have shown effects on degree of polymerization and cellulose crystallinity, applied to sludge [38,39]. Among the physico - chemical technologies there have been many reported developments on lignocellulosic materials, e.g. steam pretreatment, ammonia fibre explosion (AFEX) and liquid hot water. Purely chemical based pretreatments are often based on pH effect and solubility properties of the material. For example acids (e.g. sulphuric acid, hydrochloric acid and organic acids such as acetic acid) or alkali pretreatments (e.g. sodium hydroxide, ammonia and lime) have been focus of attention since those methods have shown best performance and includes the most promising processes for industrial applications [3,36]. Biological pretreatments uses microorganisms to treat the lignocelluloses and enhance enzymatic hydrolysis. This type of treatments are also investigated to improve biogas production by improving digestion. However this type of treatment rate is often very low and not yet applicable at large scale [40,41].

Steam Pretreatment

Steam pretreatment has the purpose of solubilizing the hemicellulose and by that expose more cellulose to the enzymatic hydrolysis and prevent formation of inhibitors (e.g. furfural, hydroxymethylfurfural (HMF), phenolic compounds) [36]. During steam treatment the biomass is exposed to steam with high pressure and consequently high temperature (e.g. 160 - 260 °C) with retention time varying from seconds up to several minutes (e.g. 20 min). The difference between steam pretreatment and explosion is that the latter has a quick depressurization and cooling down at the end, causing the water in the material to "explode". A drawback of steam explosion and steam pretreatment is the risk of condensation and precipitation of soluble lignin [36].

Diluted acid and concentrated acid

The naturally occurring enzymatic hydrolysis of the carbohydrates is, due to the lignocellulosic materials natural resistance, very slow and it is difficult to reach high sugar yields without pretreating the material. One way hydrolyse is to use acid, either concentrated or diluted. The main reaction during acid-based technique is the hydrolysis of xylan, a hemicellulose [36]. Dilute acid pretreatment can be performed for instance with sulphuric acid (e.g. 0.1 - 1 %). It can be performed at short retention time at high temperature (e.g. 5 min/180 °C) or larger time intervals and lower temperatures (e.g. 90 min/120 °C) [3]. A drawback of the diluted-acid method is that it is not specific to polymeric sugars, hydrolysing all three components, i.e. cellulose, hemicellulose, lignin. Some of the resulting hydrolysing products can be toxic to the microorganisms, such as the ones of aromatic nature. When using concentrated acid, the operation temperature can be lowered (e.g. 40 °C) but corrosion dangers are still a drawback. Inhibiting compounds, such as furfural and HMF, are also formed when concentrated acid is used, however the anaerobic microorganisms can also completely degrade both furfural and HMF and to some extent some methanogens responsible for methane production can after an acclimatization period handle those toxic compounds [42]. In contrast to the yeast commonly used for ethanol fermentation, which only can degrade the aldehydes to the corresponding alcohols, which are less toxic [36]. Another difficulty with handling high acid concentrations is the recovery of the catalyst, which has to be done due to economic reasons [3,43]. Acetic

acid (HAc) can be an alternative dilute organic acid catalyst. An advantage of the HAc is that it occurs naturally as a byproduct in the hemicellulose breakdown. HAc is consumed during the process as it is transformed to methane gas [44].

The acid acts as a catalyst and it can be added to the material by two methods: (1) impregnation or (2) spraying. By using the impregnation method the material is soaked in a liquid with the desired concentration (usually low concentration) of the catalyst and occasional excess liquid is removed after the impregnation. The spraying technique is based on the simple spraying of the catalyst onto the biomass. This requires higher catalyst concentration for the sprayed liquid as well as a temperature increase compared to the impregnation method [45].

Alkaline

Another way to improve the digestibility of lignocellulosic biomass is instead to increase the pH. At alkaline conditions the biomass solvates and saponifies, causing a swollen state that allows higher accessibility for enzymes and microorganisms [36]. This lowers the degree of polymerization of the carbohydrate polymers but it can also solubilize lignin. Compared to acid hydrolysis, the pressure and temperature necessary for this technique is slightly lower [22]. If the pH is increased excessively there is a peeling of end groups, possible degradation of dissolved polysaccharides and unwanted alkaline hydrolysis. Monomeric forms of hemicellulose for instance are probably easier transformed into inhibitors such as furfural [46]. The most common alkaline additives are: (1) sodium hydroxide, (2) potassium hydroxide (3) ammonium hydroxide and (4) lime. Lime is from an economical point of view a strong option but the formation of calcinations in the equipment is a big disadvantage [2]. Ammonium hydroxide itself must be recycled and handled cautiously to make the process environmentally more feasible and diminish the consumption of the catalyst. NaOH has received the greatest attention due to its great delignification capacity, essential to achieve high biomass digestibility [47,48].

2.7 Sudangrass

Sudangrass (*Sorghum sudanese*) is an important crop worldwide used for animal fodder, production of alcoholic beverages and biofuels. It is a part of the grass family and it native to tropical and subtropical regions (eastern Africa) however it is cultivated in many parts of the world such as southern Europe and Central America [49].

Some studies have been performed on NaOH as a catalyst coupled to sorghum. Sambusti et al. have performed several studies on sorghum. In one of those studies, it was shown that the kinetics increased with NaOH dosage but the methane yield was not affected by NaOH dosage, temperature and contact time [50]. In a second study by Sambusti et al., it was presented that NaOH soaked sorghum at 55 °C for 12 h at 4 or 10 g NaOH/100 g dry matter (DM) showed a reduction in lignin by 50-70 % [51]. Cao et al. previously showed that sweet sorghum bagasse with diluted NaOH and autoclaving gave the best hydrolysis yield and total sugar yield compared to untreated sorghum [52]. Sambusti et al. also showed that at 1 g NaOH/ 100g TS displayed a methane production increase of 14 % if compared to untreated ensiled sorghum forage (*Sorghum sudanense hybrid*) [53]. However no steam pretreatment was performed and the soaking at 40 °C/24 h rather than spraying was used.

So far the acid pretreatment of sorghum has not been studied in depth. One of the promising catalysts is acetic acid. This organic acid is a byproduct of the hemicellulose degradation, especially from agricultural residues. In the study by Bondesson et al. the corn stover was impregnated with an aqueous solution of 1 wt. % HAc for 30 minutes. It was found that HAc was a great potential pretreatment catalyst, since it solubilize xylose, a sugar that can be used to produce methane. The solid fraction was used for ethanol production and the liquid fraction for biogas production [44]. The study by Zabihi et al. investigated wheat straw soaked with acetic acid and pretreated with steam explosion. The impregnation method consisted of different concentrations (0.0, 25, 50 and 75 % v/v) and temperatures (30, 40, 50 and 60 °C) for 18 hours. The results showed that it was more effective with catalyst and steam explosion than with steam explosion alone [54].

3 Material and methods

An overview of the Material and Methods, presented as a flow chart, can be found in Appendices V.

3.1 Raw material

The IC were cultivated at Kronoslätt Farm close to Trelleborg, Sweden. It was sown the 11th of July 2014 and harvested on the 7th of November 2014. The crops had an average chopping length of 10 mm. The amount of nitrogen fertilizer used was 40 kg/ha and it was applied to the soil the 12th of July 2014. The six different IC were ensiled as well as fresh samples were taken from each crop. On the fresh material, total solids (TS) and volatile solids (VS) were measured after harvesting and thereafter frozen. When samples were to be used, the material was thawed.

The IC were ensiled with lyophilised powder of lactic acid bacteria SiloSolve MC (Chr. Hansen A/S, Hørsholm, Denmark). For ensiling different ensiling containers were used. Oilseed radish, phacelia and hemp were ensiled in 100 L barrels, which were sealed with silicon and lids. All IC (except hairy vetch) were ensiled in 1000 L intermediate bulk containers (IBC), pressed by stepping on it and then the container was covered with plastic cover and sand, in order to keep the environment anaerobic. Also hemp, oilseed radish and phacelia were ensiled in 20 L buckets and sealed with tightly fitting lids in order to keep the environment anaerobic. The pH was measured for the ensiled material, the results are presented in Appendices IV.

3.2 Steam pretreatment

The ES was pressed into a solid fraction (SF) and a liquid fraction (LF). The SF was then pretreated with different catalysts, at different temperatures and residence time, as shown in Table 1 below (see Appendix IV for schematic picture of the pretreatment). The choice of catalyst was based on previous studies, as developed below.

Steam pretreatment was performed in a pilot-scale system, explained elsewhere [55]. The catalyst solution used were 1 wt. % HAc or 2 wt. % NaOH of TS (weight percentage of total solids). The catalyst solution was sprayed onto the material and left to impregnate in a cement mixer for 30 min. Acid pretreatment was investigated at three different temperatures (180 °C, 190 °C and 200 °C) and at two different residence times (5 and 10 min). In the alkali pretreatment case, four different temperatures were investigated (180 °C, 190 °C, 200 °C and 210 °C) at a 10 min residence time. As a comparison, two samples without any catalyst were steam pretreated, the conditions for these samples were 190 °C and 10 min, respectively 210 °C and 10 min. All the investigated conditions can be seen in Table 1. After the steam pretreatment the slurry was collected from the flash cyclone and also pressed to a SF and a LF.

Table 1. Investigated conditions during steam pretreatment of SF of the ES. Acetic acid (HAc) 1 wt. % of TS and sodium hydroxide (NaOH) 2 wt. % of TS. Catalyst was sprayed onto the material and thereafter left to impregnate in a cement mixer for 30 min.

Condition	Catalyst used with steam pretreatment
180 °C 5 min	HAc
180 °C 10 min	NaOH
190 °C 5 min	HAc
190 °C 10 min	No catalyst/HAc/NaOH
200 °C 5 min	HAc
200 °C 10 min	NaOH
210 °C 10 min	No catalyst/NaOH

3.3 BMP analysis

When determining the biochemical methane potential (BMP) of the IC and the pretreated sudangrass an Automatic Methane Potential Test System (AMPTS, by Bioprocess Control, Lund, Sweden) was used. There were two experimental settings for the BMP determination of the IC, i.e. BMP 1 and BMP 2. For BMP 1 the IC quadruplicate samples were done, except for hairy vetch where only duplicates were done, due to shortage in thawed crop material. Also for BMP 1 the water bath was not filled with water between days 4-8. Therefore the samples were not incubated in the water bath at 37 °C. Due to this fault, a second BMP was performed, BMP 2. For BMP 2 the IC quadruplicate samples were done, except for sudangrass where the values presented later on are extracted from the BMP tests coupled to the pretreated material, as explained below. Two different controls were included: four samples with cellulose and inoculum and four samples with only inoculum. For the pretreated sudangrass each condition were done in quadruplicates. The pretreated material was as mentioned before pressed into a solid and a liquid fraction. Those fraction were mixed together prior the methane tests. Except for the ES which was run into separate BMP flasks, four with LF and four with the SF.

The inoculum used for all the BMP tests were taken from the same source, the sewage plant in Källby, Lund. Five days before the start of each BMP test, the inoculum was collected and incubated in a water bath at 37 °C. The BMP was determined according to the Operation and Maintenance Manual from Bioprocess Control [56]. The incubation temperature for the AMPTS was also 37 °C. Each AMPTS flask contained 300 g inoculum. For the substrate and cellulose, a ratio of 2:1 VS inoculum to VS substrate or cellulose was used. The used cellulose is 50% cellulose powder microcrystalline, MP Biomedicals, USA. The substrates tested were mixed with inoculum and thereafter incubated for at least 30 days. The methane yield is commonly related to TS or VS, it is then called the specific methane yield. The assumed methane content in the AMPTS flasks were determined with gas chromatography (GC) before shutting down

3.4 Analysis

3.4.1 Determination of total solid content and volatile solid content

TS were determined by drying the sample in an oven at 105 °C for 24 hours. The TS was then calculated with Equation 1.

$$\text{TS \%} = \frac{\text{Weight of dry sample (g)}}{\text{Weight of wet sample (g)}} \cdot 100 \quad (1)$$

VS were determined by ashing the samples in an ash oven at 550 °C for two hours. The VS was then calculated with Equation 2.

$$\text{VS \%} = \frac{\text{Weight of dry sample (g)} - \text{Weight of ashed sample (g)}}{\text{Weight of original sample (g)}} \cdot 100 \quad (2)$$

3.4.2 Determination of water insoluble solids, WIS

WIS was determined according to the National Renewable Energy Laboratories (NREL) methods [57]. This was done by washing the SF from each pretreatment condition until the filtered washed liquid was almost clear. The washed sample was then dried in 105 °C for at least 24 hours. The WIS was calculated with Equation 3 below.

$$\% \text{ WIS} = \frac{\text{Weight of dried and washed sample (g)}}{\text{Weight of original sample}} \cdot 100 \quad (3)$$

3.4.3 Composition analysis

Intermediate crops

The composition analysis of IC were determined according to the NREL protocol [58–60]. The samples were dried in 45 °C and thereafter milled. Extraction was done on all the intermediate crops.

Pretreated sudangrass

The composition analysis of the washed sample, the WIS, of SF pretreated sudangrass were determined according to the NREL protocol [58–60]. The samples were dried in 45 °C and thereafter milled. In order to confirm if either extraction combined with WIS was necessary or not for the pretreated sudangrass SF, a pretreatment condition was chosen (NaOH 180 °C 10 min) for validation. The two scenarios were (1) WIS followed by ethanol extraction and (2) only WIS. For the LF, the composition analysis was determined according to NREL protocol [61]. Calcium carbonate was used to neutralize the samples before it was analysed in a high-performance liquid chromatography (HPLC).

3.4.4 Determination of sugar content

Intermediate crops

The composition of the IC were determined according to the NREL protocol [59]. Sulphuric acid was used to convert polysaccharides to monomeric sugars. Calcium carbonate was used to neutralize the samples before it was analysed in HPLC.

Pretreated sudangrass - solid fraction

The composition of the hydrolysate of the pretreated sudangrass were determined according to the NREL protocol [59]. Sulphuric acid was used to convert polysaccharides to monomeric sugars. Calcium carbonate was used to neutralize the samples before it was analysed in HPLC.

Pretreated sudangrass- liquid fraction

The composition of the hydrolysate of the pretreated sudangrass were determined according to the NREL protocol [61]. The pH of the liquid was recorded and required amount of sulphuric acid was added to bring the acid concentration of each aliquot to 4 %. Calcium carbonate were used to neutralize the samples before it was analysed in HPLC.

HPLC

The carbohydrates (glucose, xylose, galactose, arabinose and mannose) were determined in an Aminex HPX-87P column (Bio-Rad, Hercules, CA, USA) at 85 °C with a flow rate of 0.5 mL min⁻¹ using water as eluent. All samples had been filtered through a filter with pore diameter 0.20 mm before analysis.

Ethanol, lactic acid, acetic acid, furfural, galacturonic acid and HMF were separated using an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) at 50 °C with a flow rate of 0.5 mL min⁻¹ using 5 mmol L⁻¹ sulphuric acid as eluent. All samples had been filtered through a filter with pore diameter 0.20 mm before analysis [62].

3.4.5 Determination of protein content in the intermediate crops

The protein was determined in the intermediate crops as received (materials total protein) and in the water extraction aliquot (extracted protein). In the water extraction step, explained elsewhere [60], water soluble materials may include nitrogenous materials such as protein. Part of the materials total protein is accounted twice if the protein in the water extraction aliquot is not subtracted, when the total composition is calculated and extractives are considered a component alone. Therefore the intermediate crop protein composition is given as the difference between the materials total protein and the protein present.

The protein present in the intermediate crops and in the extracted water aliquot was estimated by determining the nitrogen content in the material. The total nitrogen content is obtained using Kjeldahls method, where the samples are well mixed and acid digested prior analysis so that carbon and nitrogen in both soluble form and in particles are included [63]. Based on the nitrogen content, the protein is estimated using a nitrogen factor of 6.25 according to NREL procedures [64].

3.4.6 Correction of TS and VS content for ensiled materials

Correction for the ensiled materials TS and VS contents had to be performed. Silage might contain varied amounts of VFA, lactic acid, ammonia and alcohol which evaporates during TS measurements and should therefore be accounted. To correct the TS, steeping of the material is performed. This was done according to methods by Porter and Murray [65]. The volatilization coefficients from Porter and Murray were used since they are based on silages mainly prepared with bacterial inoculants. The steeped liquid fraction of the ensiled IC was filtered from the slurry and was investigated for acids in HPLC, (Jasco Co., Tokyo, Japan) with an Aminex HPX-87H column (Bio-Rad Laboratories Inc., Hercules, CA, USA) and a refractive index detector (Erc Inc., Huntsville, AL, USA). Sulphuric acid (5 mmol L⁻¹) was used as the mobile phase (0.6 ml/min), and the oven temperature was 40 °C.

3.4.7 Assumed methane content in the AMPTS flasks

GC tests were performed to measure the amount of methane, carbon dioxide, nitrogen and oxygen there were in the AMPTS flasks dead volume. The GC used (Varian 3350 Walnut Creek, CA, USA) was fitted with a Haysep Q 80/100 mesh column, a molecular sieve column and a thermal conductivity detector. Helium was used as the carrier gas and the column temperature was set to 70 °C, the injector temperature to 110 °C and the detector temperature to 150°C [66].

3.4.8 Extraction on pretreated sudangrass

The material to be analysed according to NREL [60], should be extracted from any compounds that might affect the analysis later on. Complete water and ethanol extraction has earlier shown to be inappropriate in steam pretreated materials (see Appendices VIII), leading to an unexpected increase of extractives in the pretreated fraction compared to the material as received. WIS was to be performed in order to wash out the material and determine the water insoluble solids for the solid fraction of the pretreated sudangrass. The water extraction step could maybe be replaced with the WIS step. In order to confirm if the material has to be washed (WIS) and thereby also extracted, one pretreatment condition was chosen (NaOH 180 °C 10 min) for investigation. The two studied scenarios were (1) WIS followed by ethanol extraction and (2) only WIS.

3.5 Severity factor

The severity factor can be a powerful tool when comparing different pretreatments. This factor gives a quantification of the harshness of steam pretreatment, and is given by Equation 4 and 5 below.

$$R_0 = t - e^{\frac{T_r - 100}{14.75}} \quad [67] \quad (4)$$

where:

- R_0 = severity
- t = retention time, minute
- T_r = reaction temperature, °C

For catalysed pretreatments, a combined severity factor $\log(CS)$ can be used, which takes the pH into account.

$$\log(CS) = \log(R_0) - pH \quad [68] \quad (5)$$

4 Results and discussion

This chapter contains the results of the thesis. The results are divided into intermediate crops and steam pretreatment. An outline of the experimental design is described in Appendices VI. Detailed results for each pretreatment can be seen in Appendices VII. The pH was measured for the LF for each pretreatment condition, the results can be seen in Appendices IX.

4.1 Intermediate crops

4.1.1 Raw material compositions

According to Table 2, hemp has the highest glucose (42.5 g of 100 g TS). Sudangrass has the highest total hemicellulose (19.7 g of 100 g TS) content, where hemicellulose is accounted as xylose, mannose, galactose and arabinose. Hairy vetch contains higher protein levels, which should be taken into account since proteins also lead to methane production. Hemp has the lowest lignin (12.8 g of 100 g TS) content while phacelia has the highest lignin content (19.7 g of 100 g TS).

Table 2 shows the total composition for each IC expressed per 100 gram TS.

Table 2. Composition of six fresh intermediate crops based on 100 gram TS. Standard deviation (SD) are based on duplicates. The acid insoluble lignin (AIL) and the acid soluble lignin (ASL) are presented as individuals, but later in the report added as the lignin content. Where no results are detected n.d.* (non-determined) is written.

Substrate	Sudangrass		White mustard		Phacelia		Oilseed radish		Hemp		Hairy vetch	
	Mean value	SD	Mean value	SD	Mean value	SD	Mean value	SD	Mean value	SD	Mean value	SD
AIL	14.6	0.4	13.9	0.4	18.6	0.7	12.1	0.4	11.9	0.8	12.3	1.9
ASL	1.4	0.4	1.1	0.1	1.1	0.09	0.9	0.04	0.9	0.1	1.1	0.08
Glucan	24.9	2.5	20.5	8.9	19.8	3.2	26.6	0.5	42.5	3.5	16.0	0.8
Xylan	14.2	2.3	7.9	3.3	7.2	1.3	9.2	0.6	7.2	0.8	3.8	0.3
Galactan	1.6	0.5	1.9	0.8	2.2	0.6	1.6	0.1	2.7	0.9	1.9	0.2
Arabinan	2.9	0.7	1.3	0.6	0.70	0.09	1.3	0.4	1.0	0.1	1.8	0.2
Mannan	1.0	1.2	1.7	1.0	2.4	0.4	2.4	0.3	2.9	0.2	1.30	0.06
Lactic acid	0.6	0.1	0.5	0.3	0.5	0.1	0.6	0.07	0.7	0.2	0.4	0.08
Acetic acid	2.1	0.7	2.8	1.3	1.8	0.9	2.7	0.7	2.6	0.4	1.5	0.2
Ethanol	0.3	0.2	0.8	0.4	0.2	0.1	n.d.*	n.d.*	2.1	1.2	0.8	0.5
Galacturonic acid	0.7	0.3	4.0	1.9	4.3	1.1	5.9	1.3	3.2	0.2	5.1	0.2
Water extractives¹	21.3	3.9	12.5	0.6	14.0	2.1	17.1	1.2	12.5	0.2	20.5	2.2
Ethanol extractives	3.2	0.3	7.7	1.2	2.8	0.07	6.5	1.0	2.6	0.3	5.5	0.2
Protein	11.9	-	10.6	-	9.4	-	10.0	-	11.9	-	25.6	-
Total	100.7		87.3		87.1		96.8		104.8		97.6	

¹ Protein amount have been reduced.

4.1.2 Methane potential/Anaerobic digestion

Figure 1 shows the results for the specific methane yield for the IC from the AMPTS equipment.

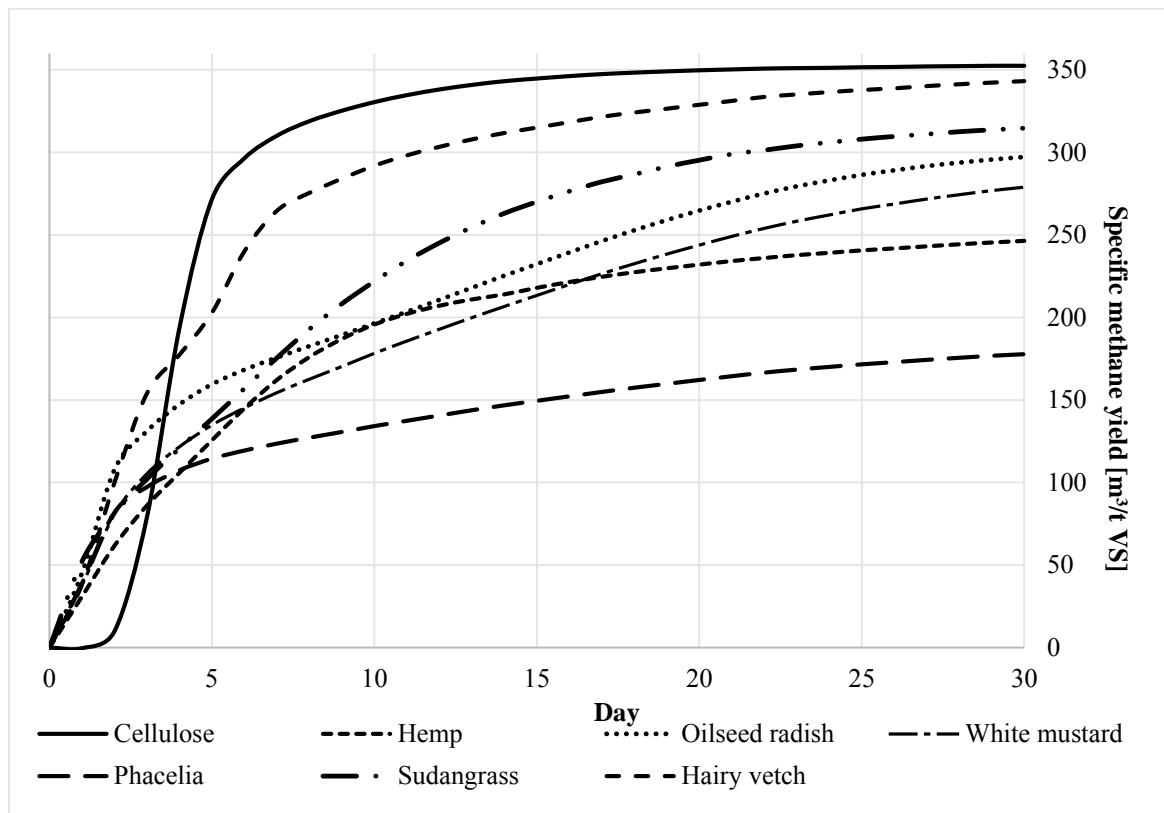


Figure 1. Accumulated specific methane yield during 30 days of batch digestion for six intermediate crops. Cellulose is included as a positive control.

As can be seen in Figure 1, phacelia have lower methane yield than the rest of the intermediate crops. This might have an explanation due to the relatively late harvest of the crop. If the crop is harvested too late, it loses dry matter and volatiles. To have an unbiased methane production comparison, phacelia might have to be harvested earlier. As can be seen in Table 2, phacelia has the highest value of AIL (18 g/ 100 g TS) which could explain the low value of methane yield. In a study by Wilson et al. showed that some cells in dicotyledons (such as hemp) lignify during aging while lignification take place in most cells in grasses (monocotyledons) [69].

For different intermediate crops, previous studies have shown that the amount of methane yield to be in a range of 250-450 m³/t VS [35,70,71]. Table 3 shows the comparison on specific methane yield of intermediate crops in earlier studies.

Table 3. Specific methane yield from previous studies.

Intermediate crop	Specific methane yield (m ³ /t VS)	Author	Reference
White mustard	239-252	Molinuevo-Salces et al.	[72]
Oilseed radish	368-450	Molinuevo-Salces et al.	[72]
Sudangrass	270-335	Sambusiti et al.	[51]
Hemp	234	Kreuger et al.	[20]
Hairy vetch (winter vetch) +oilseed rape	399-415	Molinuevo-Salces et al.	[72]
Hairy vetch (winter vetch) +triticale	ca 360	Molinuevo-Salces et al.	[72]
Hairy vetch (winter vetch) +winter ryegrass	ca 380	Molinuevo-Salces et al.	[72]

Table 4 shows the accumulate specific methane yield after 30 days for both BMP 1 and BMP 2, which also can be seen as the last data point in Figure 1 for BMP 2. The last data point for BMP 1 can be seen in the Appendices I. In Table 4 it can be seen that for BMP 1 sudangrass shows the highest specific methane yield (314 m³/ t VS) followed by hairy vetch, oilseed radish and white mustard. However white mustard has shown to be unsuitable when sown after oil-rich crops, e.g. rapeseed. This makes the establishment of white mustard specifically hard in Sweden at actual conditions [73]. When comparing BMP1 and BMP 2 from Table 4, all crops show relatively little variation, expect for hairy vetch. However the results from BMP 2 are based on four data conditions rather than only two data conditions in BMP 1. Also the accumulated specific methane yield for hairy vetch in BMP 2 is closest to literature value, according to Table 3. For now on results from BMP 2 will be used on upcoming calculations and analysis. When comparing Table 3 and 4, the sudangrass accumulated specific methane yield can be related to the earlier studies. For the oilseed radish the results in this thesis are lower than the earlier studies, but when comparing hemp and white mustard the results are higher than previous studies.

Table 4. The accumulated specific methane yield for the intermediate crops after 30 days BMP tests. BMP 1 and BMP 2 are two different analysis circumstances. The major difference is that hairy vetch for BMP 1 was analysed in duplicate rather than quadruplicate as all the other cases. Also in BMP1 the water bath was not filled regularly. The standard deviation (SD) is also presented.

Intermediate crop	Highest specific methane yield BMP 1 (m ³ / t VS)		Highest specific methane yield BMP 2 (m ³ / t VS)	
	Mean value	SD	Mean value	SD
Sudangrass	314	23	316	19
Hairy vetch	305 ¹	25	343	22
Oilseed radish	304	10	297	13
White mustard	290	32	278	11
Hemp	262	16	246	18
Phacelia	185	16	177	12

¹ Only measured in duplicates

The theoretical methane yield is calculated combining the component analysis found in Table 2 and methane yield for respective component found in Table 5. The specific methane yield for each component is calculated by a mass balance over the reaction to methane and carbon dioxide of the respective substrate, in standard temperature and pressure (STP) conditions (see Appendices II for example of the calculations). The calculations for the actual methane yield are done with the accumulated methane yield after 30 days values from anaerobic digestion BMP 2 presented in Table 4 and the substrate TS amount for each crop. The overall yield is thereafter calculated for each crop by dividing the actual methane yield with the theoretical methane yield. The result can be seen in Table 6. Note that lignin is considered to be inert due to its recalcitrant nature [27].

Table 5. Specific theoretical methane yield for substrates with known compositions. For protein, glucan, xylan, galactan and mannan the values are taken from Björnsson et al. [74]. For acetic acid, lactic acid, ethanol and galacturonic acid the values are calculated, see Appendices II. For water extractives and ethanol extractives the values are approximated by a relative estimation close to methane yield for carbohydrates and protein.

Substrate	Methane yield (L/g)
Protein	0.5
Glucan	0.415
Xylan	0.42
Galactan	0.415
Arabinan	0.42
Mannan	0.415
Acetic acid	0.405
Lactic acid	0.405
Ethanol	0.79
Galacturonic acid	0.31
Water extractives	0.45
Ethanol extractives	0.45

Table 6. Theoretical yield for the intermediate crops. The actual methane and theoretical methane are expressed in L/g TS. For the theoretical methane, 5 % have been reduced from each substrate due to the assumption that 5 % of the degradable material is used to nourish microbial growth. The theoretical methane yield is calculated combining the component analysis found in Table 2 and methane yield for respective component found in Table 5.

Intermediate crop	Actual methane (L/ g TS)	Theoretical methane (L/ g TS)	Yield (%)
Sudangrass	0.31	0.32	97
White mustard	0.26	0.27	96
Phacelia	0.15	0.25	60
Oilseed radish	0.27	0.31	87
Hemp	0.22	0.34	64
Hairy vetch	0.30	0.32	94

According to Table 6, sudangrass and white mustard already have a high yield and might not have a potential for methane production boost due to pretreatment. This results were not correctly presented when the pretreatment material was to be chosen. However this values

should not be taken as an absolute factor as the theoretical methane can be calculated by means other than component analysis. For example a bomb calorimeter could be used to calculate the theoretical energy yield of the IC and with further calculations even the theoretical methane yield can be calculated [75,76]. Note that fat is indirectly accounted for in the theoretical methane yield in the ethanol extractives.

A natural influential factor in the choice of IC for pretreatment study is relationship of the methane yield per hectare for each crop. The result can be seen in Figure 2. For data concerning the biomass yield for the different intermediate crops, see Appendices III.

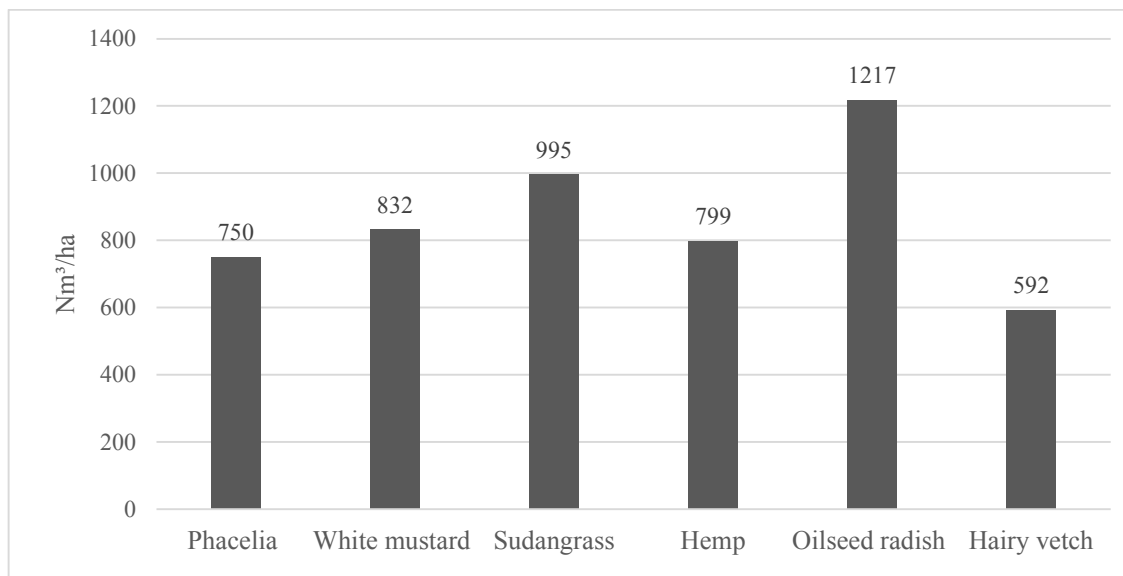


Figure 2. Specific methane yield per hectare (Nm³/ha) for the six different intermediate crops.

Figure 2 shows that oilseed radish appears to be a suitable choice due to its relatively higher yield per hectare. However in reality this crops dry matter is low (16 % TS) making the transportation a problem. The crop leaches its liquid fraction during transportation, leading to losses of material.

Taking into account the component analysis, methane yield, methane yield per hectare and other aspects such as material handling and real-life establishment of the crop, sudangrass and phacelia had the most promising potential. However the silage condition and material disposability has also to be accounted for.

To decide which material to use for the pretreatment, the different ensiling containers were examined. 20 L buckets of ensiled phacelia were opened and there were mould in the buckets, indicating that the ensiling had been compromised. The ensiled phacelia in 1000 L IBC containers, had a recognizable earth-like smell which indicated decomposition. The 100 L phacelia had a characteristic acidic smell, interpreted to be the smell of butyric acid. This acid is known to be hazardous to the human lungs [77]. Ensiling of sudangrass was performed in a 1000 L IBC container and the silage was considered a succeeded ensiling process. Silage from white mustard in 1000 L IBC container were not a choice for pretreatment due to mould in the material throughout the container. Silage of hemp were good in 20 L buckets, but there was not enough material for the investigation of pretreatment with two different catalysts or varied

pretreatment conditions. When combining all factors sudangrass was selected as the best choice for pretreatment.

4.2 Steam pretreatment

The ensiled sudangrass (ES) was therefore tested for its biochemical methane potential, represented in a liquid fraction (LF) and in a solid fraction (SF). Fresh sudangrass (FS) was also tested as a comparison. Figure 3 shows the specific methane yield for ES (SF + LF) in comparison to FS, with different bases a) tonne VS and b) tonne wet sudangrass.

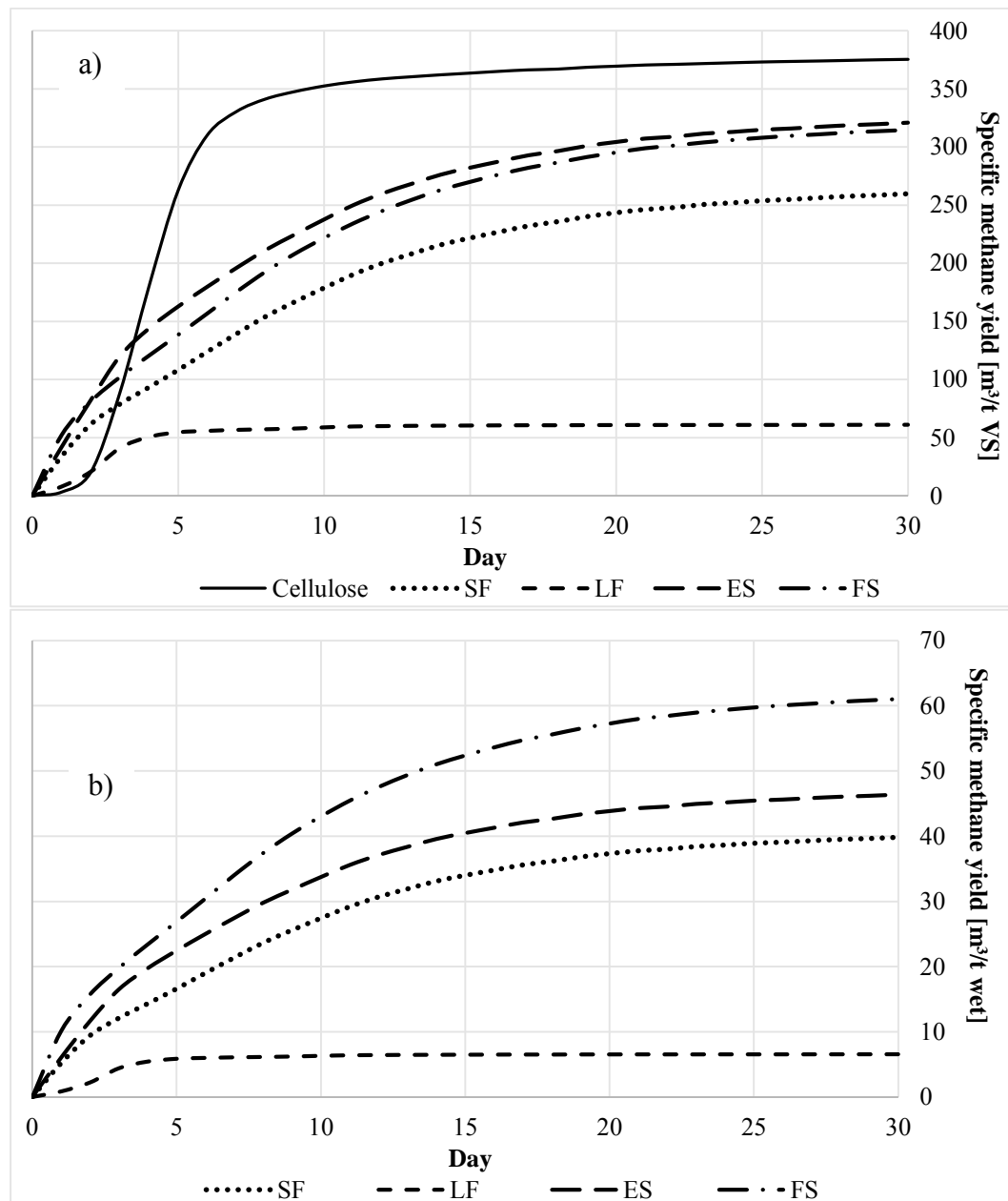


Figure 3. a) The specific methane yield (m^3/t VS) and b) the specific methane yield (m^3/t wet sudangrass) for ensiled sudangrass (ES) (which is an addition of solid fraction (SF) and liquid fraction (LF)) in comparison to fresh sudangrass (FS). SF and LF are corrected according to the pretreatment mass balance. In other words, SF's and LF's VS amounts are relative to the pressing step mass ratio.

Figure 3 a) shows the comparison between ES and FS. It is shown that the ensiling process did not affect the methane yield significantly when VS is the base. Studies have shown an increase biogas yield due to ensiling [78,79], however no TS and VS correction due to VFA's were done in those studies, leading to an overestimation of the methane yield. Other studies have shown the importance of correction of total solids, if the methane yield is to be related to TS and VS [65,80]. There is an uncertainty to the ensiling process, as the 1000 IBC container may have conceded air. There are also uncertainties to the TS and VS measurements of the ES as well as the LF and SF TS and VS measurements. Even if the wet weight before and after pressing the ES added up, the TS and VS did not. In other words, there is higher amount of both TS and VS before pressing than after separation into LF and SF. This can be caused by error in the TS and VS measurements. A comparison between Figure 3 a) and b) shows that there is a bigger gap between the specific methane yield of FS and ES in b) compared to a) where the difference is less significant. This could be explained by possible losses due to butyrate fermentation, where considerable amount of energy is lost due to the release of hydrogen [80]. In other words, the silage process might have been compromised.

4.2.1 Acetic acid

The catalyst chosen for the acid steam pretreatment was an aqueous solution of HAc (1 wt. % of TS) that was sprayed onto the ES and mixed for 30 min in a cement mixer. To spray the catalyst onto the material is considered a more feasible alternative to impregnation by soaking, where the material is soaked in a liquid with the desired concentration of the catalyst. Impregnation by soaking requires a larger water amount to be added, possibly followed by pressing of the material, which makes it industrially more costly than spraying. [81].

Methane potential/Anaerobic digestion

Material (both SF and LF in a slurry) from the four different conditions from pretreatment with HAc was run in AMPTS in quadruplicates. The accumulated methane yield after 30 days can be seen in Figure 4.

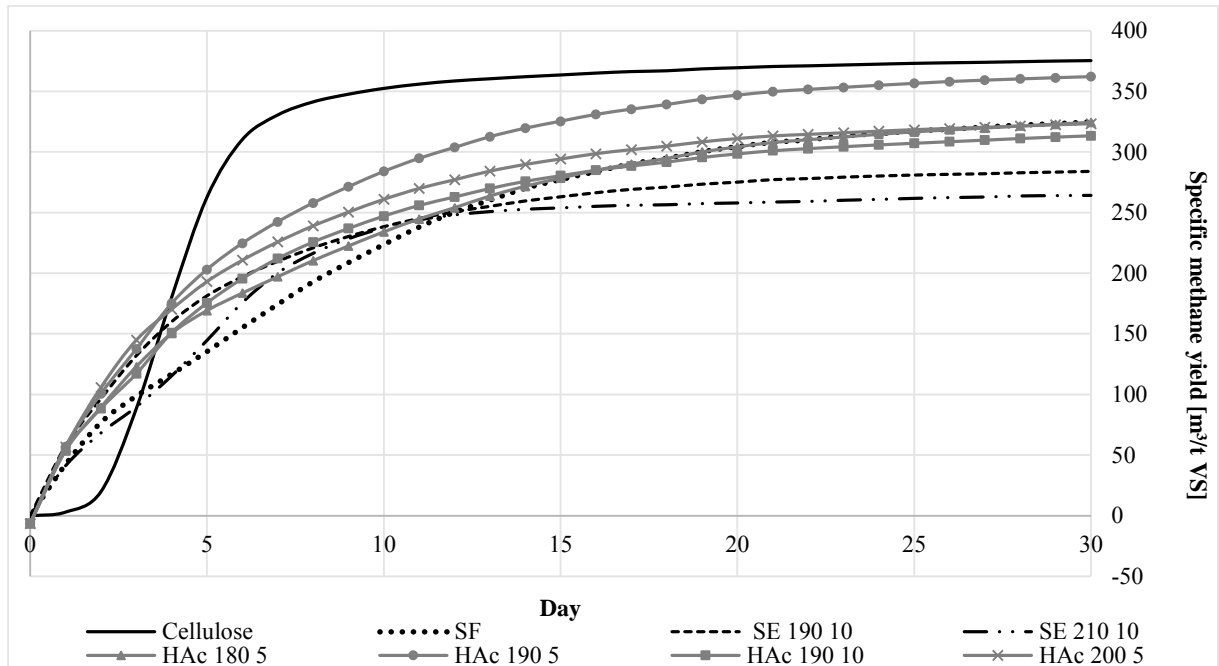


Figure 4. Accumulated specific methane yield (m^3/t VS) during 30 days of batch digestion. Cellulose is included as a positive control. The different conditions are presented with or without catalyst (steam pretreatment without catalyst (SE) and steam pretreatment with acetic acid catalyst (HAc)). Acetic acid catalyst 1 wt. % of TS, impregnation by spraying. The investigated temperatures were 180-210 °C and the residence time investigated are 5 or 10 min. The untreated solid fraction of the ensiled sudangrass (SF) is presented as a comparison. The calculated amount of methane produced from the added HAc catalyst is reduced from the four conditions, 5 % have been reduced from each HAc pretreatment due to the assumption that 5 % of the added catalyst is used to nourish microbial growth.

As can be seen in Figure 4, the condition at 190 °C and 5 min is the optimal condition in the sense that it gives the highest methane yield compared to the untreated ES SF. It causes an increase of methane yield in comparison with SF which indicates the improvement of pretreatment with HAc as catalyst. It has to be noted that 95 % of the calculated amount of methane produced from the added HAc catalyst is reduced from the four conditions, where 5 % have been reduced from each HAc pretreatment due to the assumption that 5 % of the added catalyst is used to nourish microbial growth. The three other conditions with HAc resulted in a lower methane yield than SF, this may be explained by rather tough or mild pretreatment conditions. During severe pretreatment conditions it will cause great degradation of hemicellulose sugars into inhibitory components, and for mild pretreatment conditions the opposite will happen (less solubilisation of hemicellulose sugars). The ideal pretreatment condition will hydrolyse the hemicellulose to its monomer sugars without further degradation to inhibitory components [82].

During pretreatment with acid catalyst the main reaction is hydrolysis of hemicellulose into xylose. But during the pretreatment the solubilised sugars can also be degraded to furfural and HMF, and further to formic acid and levulinic acid. Furfural and HMF can have toxic effect on methanogens. The degradation of the sugars is more extensive the more severe (higher temperature and lower pH) the pretreatment is [83]. However both furfural and HMF can also be completely degraded by anaerobic microorganisms [84].

In Table 7 the accumulated specific methane yield after 30 and 10 days for each condition is presented. This in order to compare the results from Figure 4 more clearly.

Table 7. Accumulated specific methane yield (m³/t VS) for each condition with acetic acid (HAc) as catalyst for AD after 10 respectively 30 days. Comparison to solid fraction (SF) is also provided.

Condition	Specific methane yield	Specific methane yield
	10 days	30 days
HAc 190 5	284	362
SF	224	325
HAc 180 5	234	324
HAc 200 5	261	323
HAc 190 10	247	313
SE 190 10	238	284
SE 210 10	237	264

Table 7 shows the specific methane yield for the acetic acid pretreated SF of ES for AD after 10 respectively 30 days. It shows that after 10 days the best condition is HAc 190 5, which also is the best condition after 30 days. After 10 days the specific methane yield for untreated SF is lower than any other condition. However this does not apply after 30 days, where only the condition HAc 190 5 is above the untreated SF. Table 7 shows that the five conditions below SF which showed lower methane yield after 30 days, have produced after 10 days 72-90 % of the methane yield detected after 30 days in AMPTS, while SF only have produced 69 % of its 30 days value.

Composition of material

Figure 5 is presented to compare the lignin, sugars and byproducts before and after pretreatment. The values are expressed as grams per 1 kg TS ES input to the pretreatment. The mass balance over the pressing and pretreatment steps was taken into consideration. The HAc catalysed material is compared to both FS and ES.

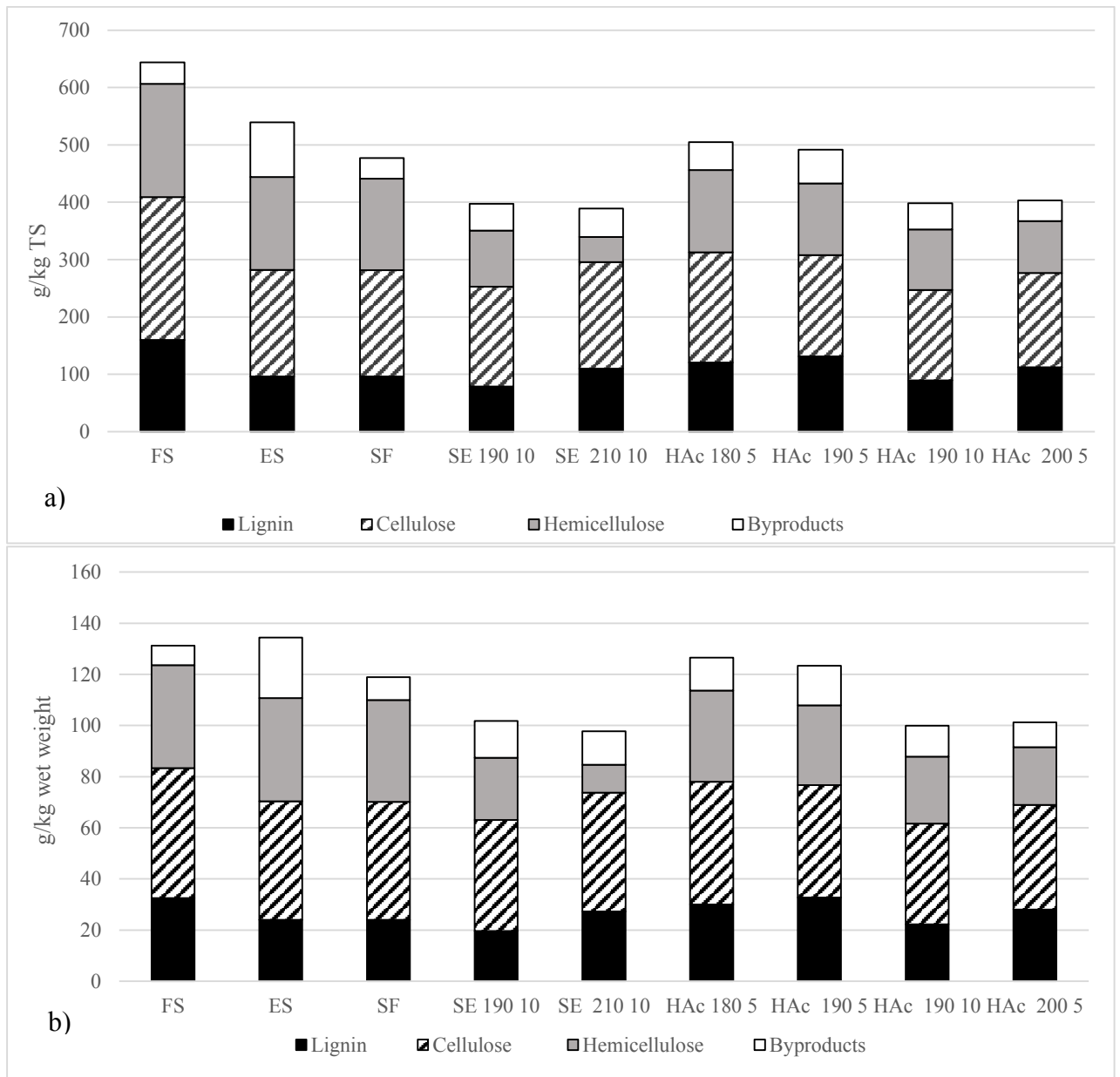


Figure 5. a) Composition of structural carbohydrates, lignin and byproducts as g per 1 kg TS sudangrass silage input to the steam pretreatment scheme. b) Composition of structural carbohydrates, lignin and byproducts as g per 1 kg wet weight of sudangrass input to the steam pretreatment scheme. The different conditions are presented with or without catalyst (steam pretreatment without catalyst (SE) and steam pretreatment with acetic acid catalyst (HAc)). Acetic acid catalyst 1 wt. % of TS, impregnation by spraying. The investigated temperatures are 180 - 210 °C and the residence time investigated are 5 or 10 min. The fresh sudangrass (FS) and the ensiled sudangrass (ES) are presented as a comparison and the solid fraction of the ensiled sudangrass (SF) is presented for comparison to the steam pretreatment.

When comparing the FS and ES in Figure 5 a) and b) it can be seen that the amount of lignin decreases when ensiling the sudangrass, during silage it should not decrease. Also the overall composition between FS and ES should be the same since nothing will happen to the material during the silage process, only converting the cellulose to byproducts, e.g. acetic acid. In Figure

5 b) the overall composition between FS and ES are quite the same, but this is not the case in a). This could be explained by the measurements of TS and VS of the silage are incorrect.

Figure 5 also shows that when comparing the lignin amount of the steam pretreatment as a comparison to the SF, it can be seen that steam pretreatment solubilize more lignin for all conditions except for the conditions with 190 °C and 10 min.

During steam pretreatment some parts of the hemicellulose hydrolyse and form acids, such as levulinic acid and formic acid. These acids can then catalyse the hydrolysis of polymeric hemicellulose and soluble hemicellulose oligomers into monomers [36], the difference between monomeric and oligomeric sugars are presented in Figure 6 further down in the report.

Previous studies show that the cellulose is not degraded when having steam pretreatment with temperatures lower than 210 °C and 10 min retention time. At temperatures around 220 °C the results show degradation of cellulose [25]. Therefore in the conditions investigated in this thesis, it should not be expected that cellulose content would vary. Figure 5 supports that theory to some extent, as the cellulose amount generally does not change. As mentioned before, there could have been some mistakes in the TS and VS calculation of the ES, leading to the gap between FS and ES seen in Figure 5. As the composition ratio of carbohydrates, lignin and byproducts may vary between FS and ES, the total mass should not differ as much as no solids are not expected to be consumed unless the silage process has been compromised.

Figure 6 present the sugar concentrations of monomeric and oligomeric sugars in the liquid fraction after steam pretreatment.

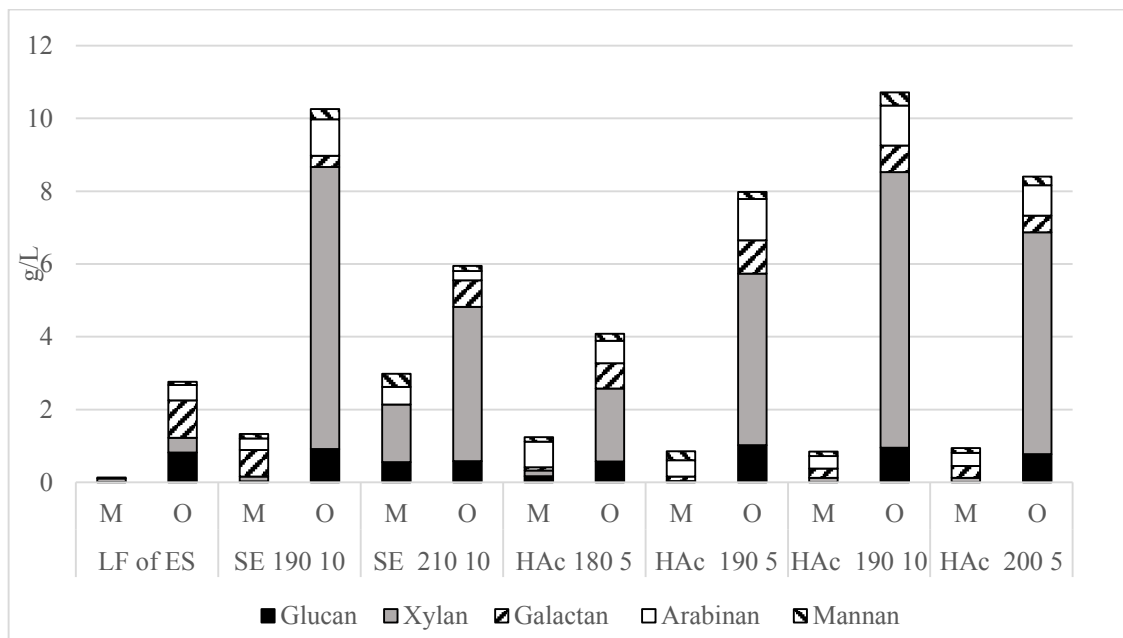


Figure 6. Concentrations of monomer (M) and oligomer (O) in $g L^{-1}$ detected in the liquid fraction. The different conditions are presented with or without catalyst (steam pretreatment without catalyst (SE) and steam pretreatment with acetic acid catalyst (HAc)). Acetic acid catalyst 1 wt. % of TS, impregnation by spraying. The investigated temperatures are 180 - 210 °C and the residence time investigated are 5 or 10 min. The liquid fraction of the ensiled sudangrass (ES) is presented for comparison.

As can be seen in Figure 6, the oligomeric sugars are detected at much higher concentrations than the monomeric sugars. For glucan, galactan, arabinan and mannan the concentration vary from 0 - 1.5 g/L but for xylan a much higher concentration is detected up to 8.0 g/L. When pretreating with acid the main reaction occurring is the hydrolysis of hemicellulose and especially the xylose. Figure 6 shows that steam pretreatment has to some extent performed what is was expected to do: to solubilise hemicellulose. The solubilized hemicelluloses, partially represented in Figure 6 by the oligomers, form hydrolytic reactions which form monomers, furfural and HMF [36].

Figure 7 shows the byproducts for ES and after SE with and without acetic acid.

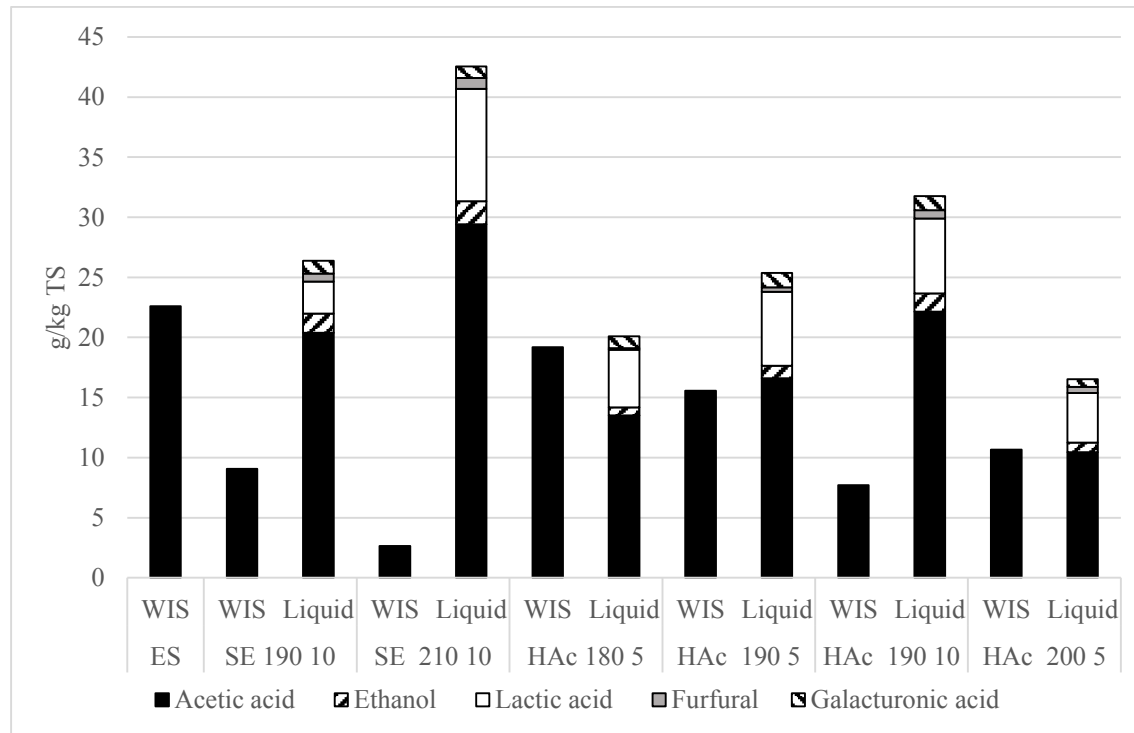


Figure 7. Composition of byproducts in WIS and liquid fraction for sudangrass silage prior and after steam pretreatment with/without catalyst, expressed as g per 1 kg TS sudangrass silage input to the steam pretreatment scheme. The different conditions are presented with or without catalyst (steam pretreatment without catalyst (SE) and steam pretreatment with acetic acid catalyst (HAc)). Acetic acid catalyst 1 wt. % of TS, impregnation by spraying. The investigated temperatures are 180 - 210 °C and the residence time investigated are 5 or 10 min. The ensiled sudangrass (ES) is presented for comparison.

Figure 7 shows that the amount of byproducts in the LF generally increases with increased temperature and increased retention time, except for the condition HAc 200 5 where the byproducts in the LF are lower than for example, HAc 190 5. When comparing the byproducts in the WIS it can be seen that the amount of HAc decreases with increasing temperature and increasing residence time. This can be explained by that the acetate is released from the hemicellulose with increasing severity, but at too high severity the acetate is further degraded.

The higher specific methane yield for the HAc 190 5 in comparison to HAc 190 10 may be explained by the results from Figure 5-7. The condition HAc 190 10 should have a better methane yield if the higher amount of accessible acetic acid detected in the LF (Figure 7) and

the higher amount of accessible xylose detected (in Figure 6) are taken into account. But that is not the case. A probable explanation could be given by Figure 5, where the amount of detected lignin is lower for HAc 190 10. At longer retention times, lignin can further degrade to byproducts that might be inhibitory to the microorganisms responsible for methane production [85].

4.2.2 Sodium hydroxide

The catalyst chosen for the alkaline steam pretreatment was an aqueous solution of NaOH (2 wt. % of TS). The solution was sprayed onto the ES and mixed for 30 min in a cement mixer. To spray the catalyst onto the material is considered a more feasible alternative to impregnation by soaking, where the material is soaked in a liquid with the desired concentration of the catalyst. Impregnation requires a larger water amount to be added, possibly followed by pressing of the material, which makes impregnation industrially more costly than spraying. [81].

The material to be analysed according to NREL [60], should be extracted from any compounds that might affect the analysis later on. Complete water and ethanol extraction has earlier shown to be inappropriate in steam pretreated materials (see Appendices VIII), leading to an unexpected increase of extractives in the pretreated fraction compared to the material as received. In order to confirm if the material has to be washed (WIS) and thereby also extracted, one pretreatment condition was chosen (NaOH 180 °C 10 min) for investigation. The two studied scenarios were (1) WIS followed by ethanol extraction and (2) only WIS. The results can be seen in Table 8. A generalization of the results of Table 8 is that slightly more lignin (AIL and ASL) and sugars are detected when no ethanol extraction is performed. Based on the results from Table 8 it was assumed that WIS alone was enough for elimination of unwanted extractives prior analysis due to the fact that the difference was not substantially great [60].

Table 8. Composition of structural carbohydrates and lignin for the solid fraction of ensiled sudangrass (2 wt. % of TS impregnated with sodium hydroxide by spraying, steam pretreatment 180 °C 10 min). Expressed as g per 1 kg TS ensiled sudangrass input to the steam pretreatment scheme. The solid fraction is either washed (WIS is performed according to NREL procedures [57]), and thereafter ethanol extracted (according to NREL procedures [60]) or only WIS is performed on the material.

	WIS + Ethanol extraction (g/ kg TS)	WIS (g/ kg TS)
AIL	54.5	66.2
ASL	4.3	2.3
Glucose	124.1	143.3
Xylose	87.6	87.5
Galactose	0.0	0.0
Arabinose	6.7	13.8
Mannose	1.5	0.0

Methane potential/Anaerobic digestion

Material slurry from the four different conditions pretreatment with NaOH were tested in the BMP tests in quadruplicates, together with the two materials which were only steam pretreated, SE. The result after 30 days can be seen in Figure 8. The SF was also BMP tested for comparison purposes.

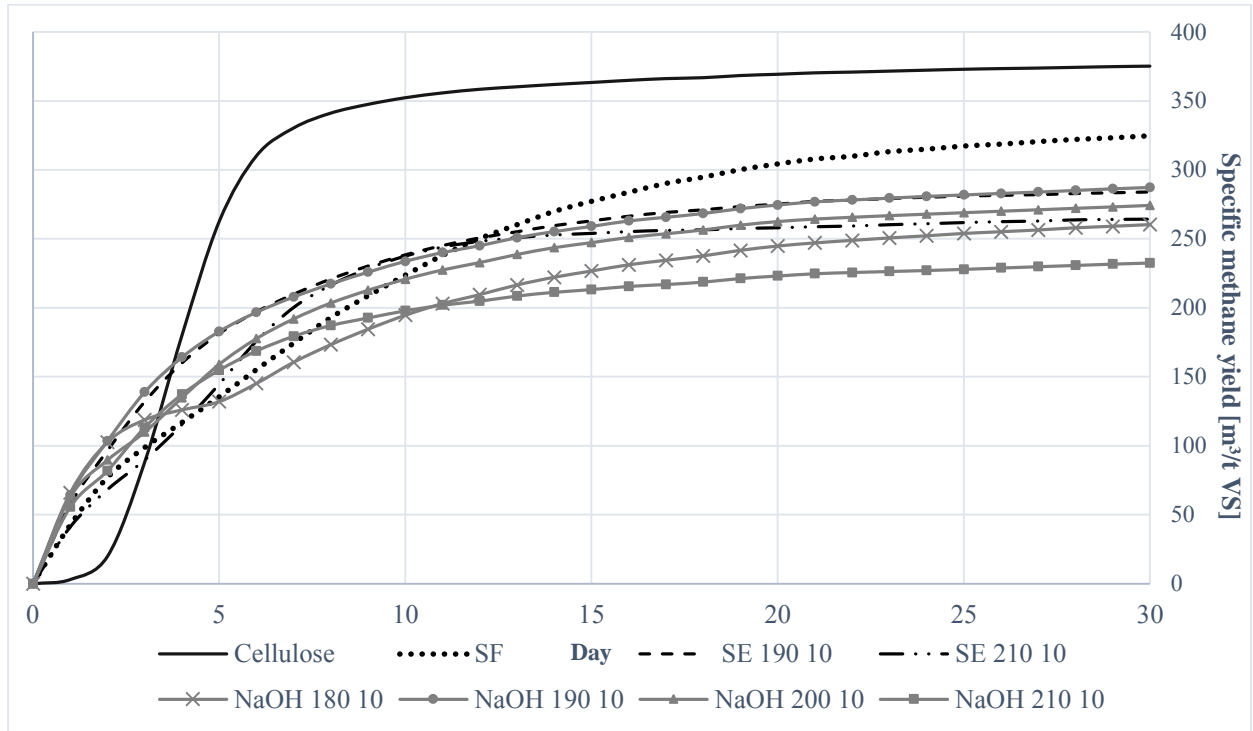


Figure 8. Accumulated specific methane yield during 30 days of batch digestion. Cellulose is included as a positive control. The different conditions are presented for the solid fraction (SF) of the ensiled sudangrass (ES) with or without catalyst (steam pretreatment without catalyst (SE) and steam pretreatment with sodium hydroxide catalyst (NaOH)). Catalyst is NaOH 2 wt. % of TS, impregnated by spraying. The investigated temperatures are 180 - 210 °C and the residence time investigated is 10 min.

According to Figure 8 NaOH does not cause an increase in methane production compared to the untreated ensiled SF. SE alone shows a decrease in methane potential as well. This could be due to the long residence time (10 min) in the SE causing condensation and precipitation of soluble lignin, even though this might not explain the scope of the decrease. The compounds produced during the condensation and precipitation of lignin are of often of phenolic nature and may have an inhibitory effect on the microorganisms responsible for methane production [36]. A local optima for both thermal and alkali-thermal pretreatment are shown at an intermediate temperature (190 °C) while higher temperatures, e.g. NaOH 210 °C show a decrease in methane production as high as 28 % compared to SF. It is known that at concentrations above 8 g/L Na⁺ is inhibitory to the AD [55]. The actual NaOH concentration due to catalyst addition should not be above 0.5 g/L, nonetheless the inhibitory factor could be a reason to the low methane yield as showed on rapeseed and sunflower by Antonopoulou et al. [83,86]. Also the inoculum might add some contribution to the sodium ion concentration.

Although the intention was to create an alkaline environment and a high pH was not achieved. This is partially due to the fact that spraying as impregnation method was used, but also possibly

due to the amount of catalyst added. For instance, ensiled LF increased its pH from 4.60 to 5.04 when unpretreated LF is compared to LF NaOH 190 10. Appendices IX present pH for the liquid fraction for all pretreatment conditions. In a study performed by Sambusti et al., 1 g NaOH/ 100g TS showed a methane production increase of 14% if compared to untreated ensiled sorghum forage (*Sorghum sudanense hybrid*). However no steam pretreatment was performed and the soaking at 40 °C/24 h rather than spraying was used. In this study by Sambusti et al. the pH after 1 g NaOH/ 100g TS pretreatment was around 7-8 [50], compared to the 5.04 mentioned above. Other studies have shown a combination of thermal-alkaline pretreatment (NaOH 0.1-2 % w/w at 125 °C/1 h) caused a decrease on soluble carbohydrates and methane yield in fresh sweet sorghum (*Sorghum bicolor L. Moench*). However in the same study thermal treatment alone caused an slight increase in methane yield [83].

In Table 9 the accumulated specific methane yield after 30 and 10 days for each condition is presented. This in order to compare the results for Figure 8 more clearly.

Table 9. Accumulated specific methane yield (m³/t VS) for each condition with sodium hydroxide (NaOH) as catalyst for AD after 10 respectively 30 days. Comparison to solid fraction (SF) is also provided.

Condition	Specific methane yield 10 days	Specific methane yield 30 days
SF	224	325
NaOH 190 10	234	287
SE 190 10	238	284
NaOH 200 10	221	274
SE 210 10	237	264
NaOH 180 10	195	260
NaOH 210 10	198	233

Table 9 shows the specific methane yield for AD after 10 respectively 30 days. It shows that after 10 days the condition with the highest methane yield is SE 190 10, closely followed by SE 210 10. The last condition that shows higher methane yield compared to SF after 10 days is NaOH 190 10, which is the condition that after 30 days gives the highest methane yield after SF. Table 9 shows that the conditions with NaOH as catalyst has after 10 days produced between 75-85 % of the final specific methane yield detected at 30 days. The conditions without catalyst (SE) produced between 84 and 90 % of the final yield at 30 days. SF produced 69 % of its highest value after 10 days and the production of methane for SF is more intense in the last 20 days than the other conditions.

Figure 9 compares the amount of lignin, cellulose, hemicellulose and byproducts before and after steam pretreatment, with and without catalyst. Both fresh and ensiled sudangrass is presented as comparison.

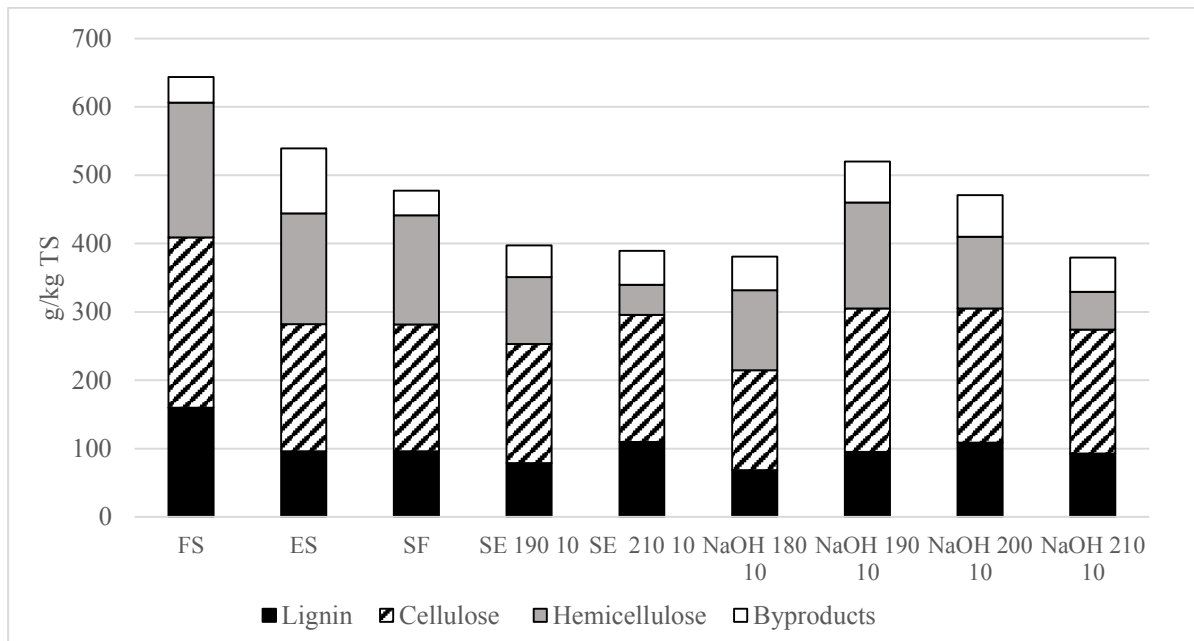


Figure 9. Composition of materials as g per 1 kg TS ensiled sudangrass (ES) input to the steam pretreatment scheme. The different conditions are presented with or without catalyst (steam pretreatment without catalyst (SE) and steam pretreatment with sodium hydroxide catalyst (NaOH)). Catalyst is NaOH 2 wt. % of TS, impregnated by spraying. The investigated temperatures are 180 - 210 °C and the residence time investigated is 10 min. The fresh sudangrass (FS) and the ensiled sudangrass (ES) are presented as a comparison and the solid fraction of the ensiled sudangrass (SF) is presented for comparison to the steam pretreatment conditions.

One of the effects expected when steam pretreating a substrate is the solubilisation of hemicellulose. According to Figure 9, the hemicellulose has not shown major changes in relative compositions in any of the pretreatments. This could be interpreted as no further degradation of the carbohydrates to byproducts take place, at least not to an extended level. Figure 9 also shows that the lignin content has shown to be generally constant, pretreated and SF being compared. An exception is found at SE 210 10 and NaOH 200 10 where more lignin is detected. The severity of these pretreatments can have caused the lignin apparent molecular mass to increase due to condensation of other components along with the lignin, leading to an unexpected increase in lignin composition [87]. The alkali pretreatment is expected to alter the structure of lignin, solubilize hemicelluloses and increase accessibility of cellulose by causing a swollen state in the structure [53]. However the pH achieved after the NaOH pretreatment (see Appendices IX) is not at alkaline conditions.

Figure 10 presents the sugar concentrations of monomeric and oligomeric sugars in the liquid fraction after steam pretreatment. Figure 11 show the byproducts for ensiled sudangrass without and after steam pretreatment with/without NaOH as catalyst.

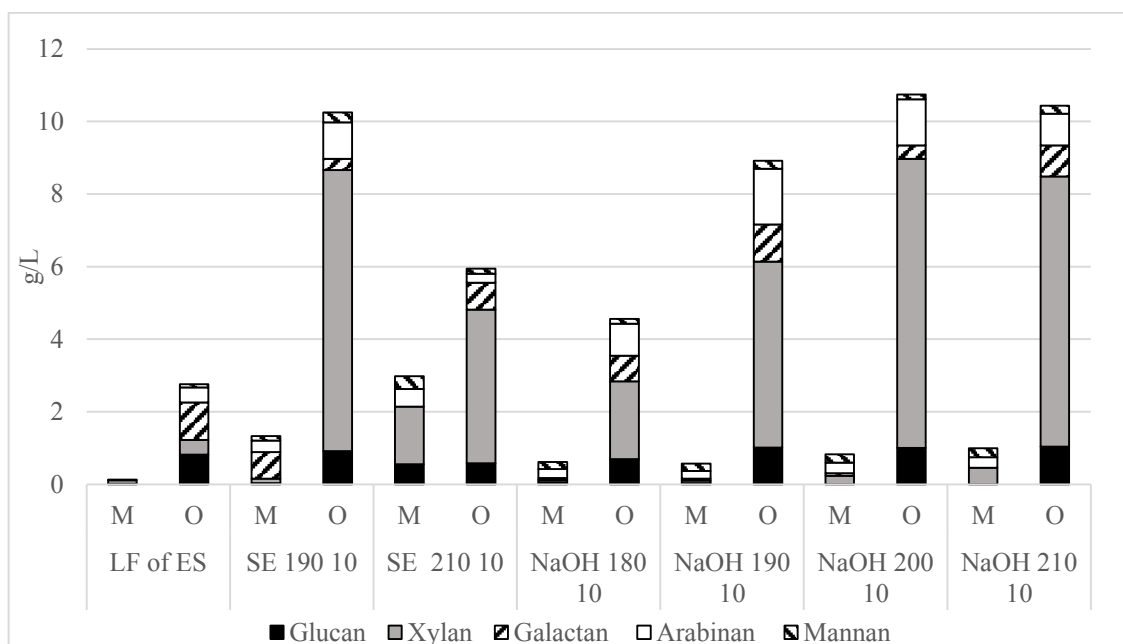


Figure 10. Concentrations of monomer (M) and oligomer (O) sugars in $g L^{-1}$ detected in the liquid fraction. The different conditions are presented with or without catalyst (steam pretreatment without catalyst (SE) and steam pretreatment with sodium hydroxide catalyst (NaOH)). Catalyst is NaOH 2 wt. % of TS, impregnated by spraying. The investigated temperatures are 180 - 210 °C and the residence time investigated is 10 min. The liquid fraction (LF) of the ensiled sudangrass (ES) is presented as a comparison.

Figure 10 shows a general increase in monomeric sugars in the liquid phase for the NaOH pretreated material, when compared to ES. This followed by an increase in concentration of oligomers, especially xylan. This proves that the solubilisation of hemicellulose was to some extent successful.

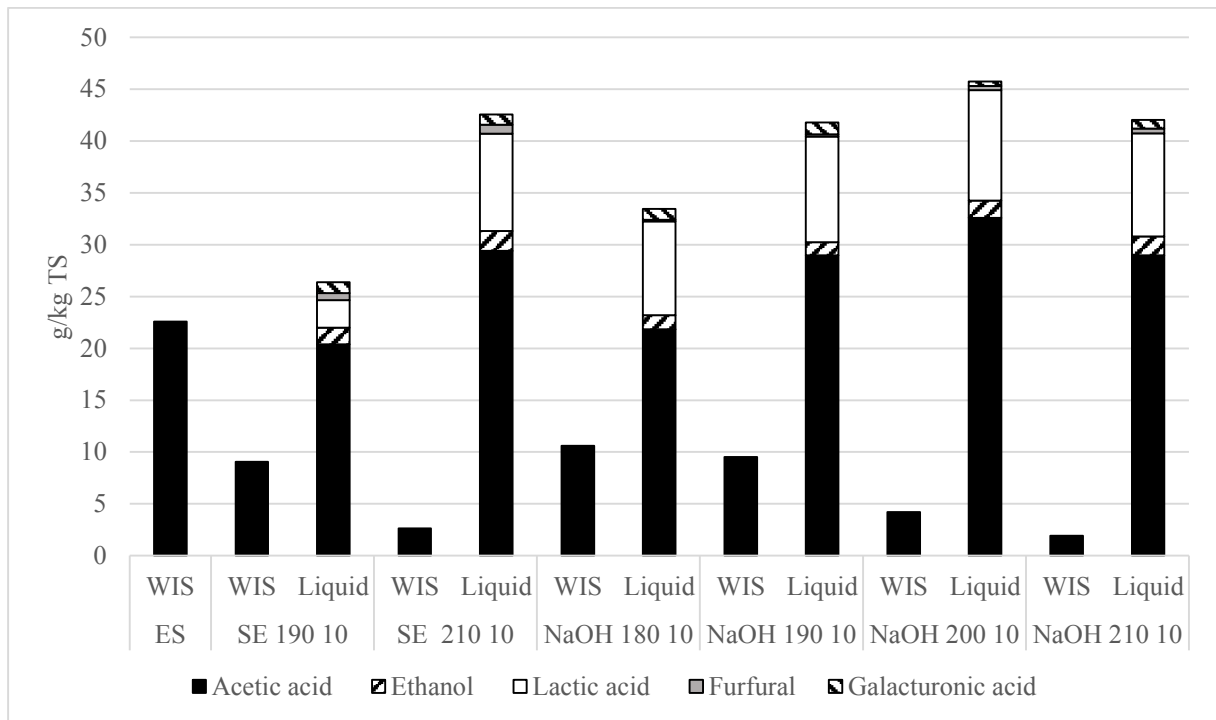


Figure 11. Composition of byproducts in WIS and liquid for sudangrass silage prior and after steam pretreatment with/without catalyst, expressed as g per 1 kg TS sudangrass silage input to the steam pretreatment scheme. The different conditions are presented with or without catalyst (steam pretreatment without catalyst (SE) and steam pretreatment with sodium hydroxide (NaOH)). Catalyst is NaOH 2 wt. % of TS, impregnated by spraying. The investigated temperatures are 180 - 210 °C and the residence time investigated is 10 min. The ensiled sudangrass (ES) is presented as a comparison.

As shown in Figure 11 there is a low concentration of furfural in every LF, which likely comes from the degradation of the pentose-type sugars. There is a clear overall increase on VFA (in this case represented as acetic acid) and lactic acid due to pretreatment when ES and pretreated sudangrass are compared. The losses in the flash step of those volatile components during the steam pretreatment are however not taken into account. HMF is not shown since the values were under the detection limits.

4.2.3 Combined results

The specific methane yield after steam pretreatment for each condition investigated for 30 days of batch digestion is presented with the standard deviation (standard error of the mean) in Figure 12.

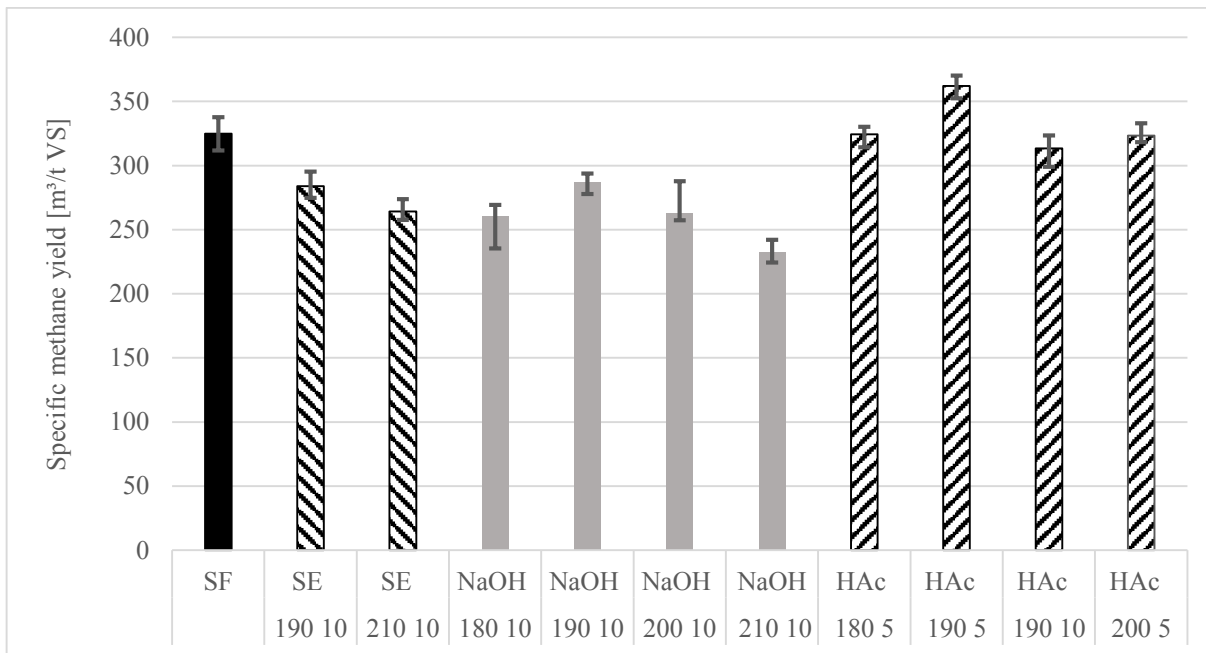


Figure 12. The specific methane yield after steam pretreatment with/without catalyst after 30 days of batch digestion, presented with the standard deviation (standard error of the mean). The different conditions are presented with or without catalyst (steam pretreatment without catalyst (SE), steam pretreatment with acetic acid (HAc) and steam pretreatment with sodium hydroxide (NaOH)). The investigated temperatures are 180 - 210 °C and the residence time investigated are 5 and 10 min. The solid fraction (SF) is presented as a comparison.

From Figure 12 it can be seen that the specific methane yield is only significantly increased when comparing to untreated SF is the HAc 190 5 condition. It can be determined that the conditions without catalyst (SE) and with NaOH catalyst gives a significantly lower specific methane yield than SF.

Figure 13 shows a comparison between the acid and the alkaline catalyst. The severity factor is plotted against the accumulated specific methane yield after 30 days.

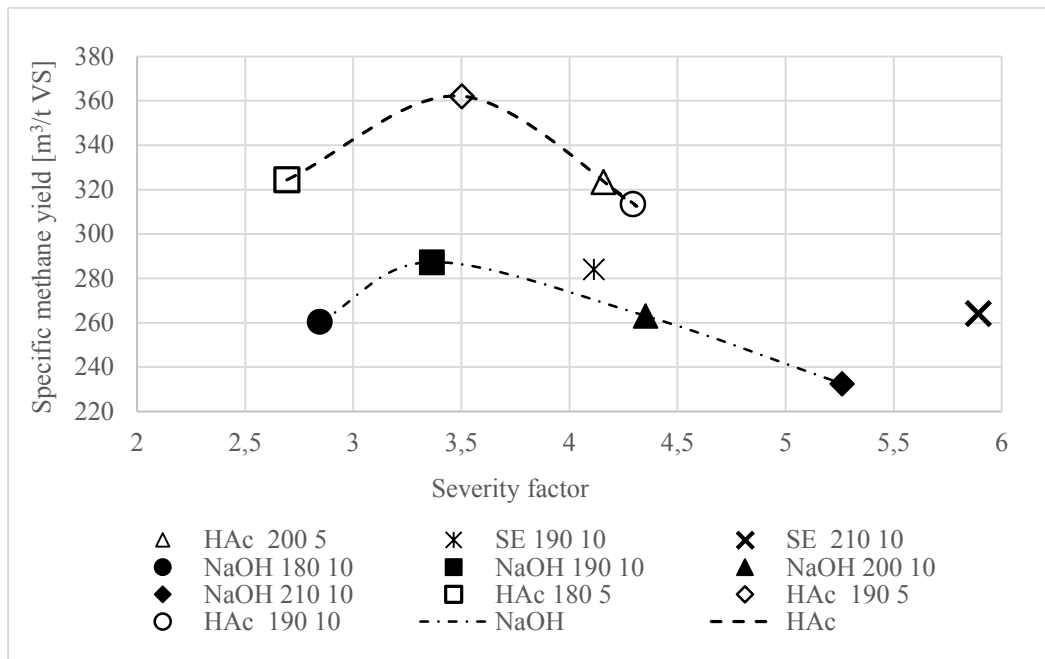


Figure 13. The severity factor (SC) with the specific methane yield after steam pretreatment with/without catalyst. The different conditions are presented with or without catalyst (steam pretreatment without catalyst (SE), steam pretreatment with acetic acid (HAc) and steam pretreatment with sodium hydroxide (NaOH)). The investigated temperatures are 180 - 210 °C and the residence time investigated are 5 and 10min.

Figure 12 shows that both acetic acid and sodium hydroxide pretreatment show a peak at 190 °C despite the different retention time. However the NaOH specific methane yield for NaOH is lower than for HAc.

5 Conclusions

From the IC: (1) oilseed radish has the highest specific methane yield per hectare, (2) hairy vetch has the highest accumulated specific methane yield after 30 days, (3) hemp has the best room for improvement when comparing actual and theoretical methane yield. White mustard had relatively good overall characteristics but share diseases with for example rapeseed, while oilseed radish is hard to transport due to the out leaching of its liquid fraction. Sudangrass has the second best specific methane yield per hectare and also second highest accumulated methane yield after 30 days. Also sudangrass was established to have undergone the best ensiling process and most material was available for analysis. Sudangrass was therefore chosen as the crop with the best potential for further pretreatment. However the ensiling process of sudangrass seemed to be compromised. This conclusion was drawn when comparing the specific methane yield per VS and for wet sudangrass. The difference between FS and ES in those two cases point to the fact that some losses could have taken place during ensiling.

The second major aim of this thesis was to evaluate the importance of pretreating a lignocellulosic material. When discussing the importance of the steam pretreatment, pretreating sudangrass gave some interesting results. The SF of the ensiled sudangrass steam pretreated and with added acetic acid 1 wt. % of TS at 190 °C and 5 min was the only condition that gave a significantly higher specific methane yield compared to the untreated SF. A conclusion for the steam pretreatment with sodium hydroxide is that this pretreatment, under the conditions investigated, did not increase the methane yield when comparing it to the SF of the ES. When the severity of the pretreatment correlated to the accumulated specific yield is taken into account, one could draw the conclusion that temperatures above 200 °C and below 180 °C should be avoided. The local optima for every pretreatment appeared to be around 190 °C. However the catalyst amount and the impregnation method chosen for this master thesis might not have been close to give the best yield conditions one could achieve.

As mentioned previous in this master thesis, it was rather difficult to correlate the component analysis to the specific methane yield. It would be of interest to compare the component analysis of untreated and pretreated material in order to find key factors that might have caused a pretreatment to be efficient or not. A pattern for the conditions could however not be drawn from the pretreatment with or without acetic acid and sodium hydroxide. It may not be possible to correlate the component analysis with the specific methane yield. Instead it might be of interest to just look at the specific methane yield, the theoretical methane possible to determine if pretreatment is viable and make an educated guess on which steam pretreatment is the best.

Conclusively it could be said that all the IC have a potential for methane production, given other aspects such as harvest time and transport are optimized for its usage. When discussing pretreatment, it is clear that there is a potential in increasing the methane outcome, giving the material has not yet reached its best yield. However it is important to keep in mind that the optimal pretreatment for a material should be systematically tested and this condition might be valid only to the material studied.

6 Future work

There are further investigations which are interesting to do in the future. The TS, VS and steeping of the ensiled material and the SF of ES could be repeated to assure that this data are measured correctly. The TS and VS seemed to be a source of error when the mass balance of the components did not add up when comparing FS, ES and pretreated ES.

Another aspect to investigate is the impregnation method for the catalyst to the steam pretreatment. Previous studies looked at the soaking impregnation method, instead of spraying the catalyst onto the material. This method showed better methane yield and it would be interesting to both look at the impregnation of acetic acid and the sodium hydroxide to see if there are improvements on the methane yield.

Further investigation of the sodium hydroxide pretreatment would be to titrate the ensiled material and to look at how much base is needed to neutralize the acids in the silage and increase the pH so an alkaline environment is achieved. Also the theoretical methane potential could be calculated through bomb calorimetry for instance, rather than by composition analysis of carbohydrates and others methane yielding components.

Since the 190 °C pretreatments showed an optima it would be interesting to pretreat sudangrass with only steam pretreatment at 190 °C and 5 min without catalyst. For the other IC, hemp would be interesting to investigate for soaking pretreatment with sodium hydroxide. Hemp showed a good potential for methane yield potential and has yet much to be tested on the alkaline catalyst front.

The idea of bio refinery could be investigated. Combing ethanol, methane and hydrogen production appears to be a promising alternative. The liquid fraction could be investigated for hydrogen tests and the pretreated ensiled sudangrass could be investigated for ethanol fermentation. Finally, it would be interesting to investigate the efficiency of the pretreatment of sudangrass, to look if it is economically possible to achieve in a full-size biogas plant. For example, if HAc catalyst at 190 °C and 5 min is to be used, about 79 % of the methane is already recovered after only 10 days. This means that if the remaining 21 % methane can be ignored, a smaller digester can be built or higher material flow can be used. An economical assessment could be performed on rather waiting the 30 days is more feasible than cutting the digestion time to 10 days.

7 Reference list

- [1] C. Gissén, T. Prade, E. Kreuger, I.A. Nges, H. Rosenqvist, S.E. Svensson, et al., Comparing energy crops for biogas production - Yields, energy input and costs in cultivation using digestate and mineral fertilisation, *Biomass and Bioenergy*. 64 (2014) 199–210. doi:10.1016/j.biombioe.2014.03.061.
- [2] P. Alvira, E. Tomás-Pejó, M. Ballesteros, M.J. Negro, Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review, *Bioresour. Technol.* 101 (2010) 4851–4861. doi:10.1016/j.biortech.2009.11.093.
- [3] M.J. Taherzadeh, K. Karimi, Pretreatment of Lignocellulosic Wastes to Improve Ethanol and Biogas Production: A Review, 2008. doi:10.3390/ijms9091621.
- [4] M. a Carriquiry, Du, *Second-Generation Biofuels Economics and Policies*, (2010) 55.
- [5] Wikipedia, Second-generation biofuels, (n.d.). http://en.wikipedia.org/wiki/Second-generation_biofuels (accessed February 5, 2015).
- [6] Swedish Energy Agency, *Energy in Sweden 2013*, (2013) 115.
- [7] Energimyndigheten, *Energiläget 2011*, (2011).
- [8] T. Lönnqvist, S. Silveira, A. Sanches-Pereira, Swedish resource potential from residues and energy crops to enhance biogas generation, *Renew. Sustain. Energy Rev.* 21 (2013) 298–314. doi:10.1016/j.rser.2012.12.024.
- [9] The European Commission, *EU Energy Markets in 2014*, 2014. doi:10.2833/2400.
- [10] Biofuel STP, (n.d.). <http://www.biofuelstp.eu/cellulosic-ethanol.html#canada> (accessed February 5, 2015).
- [11] Swedish Biogas International, Swedish Biogas International, (2013). <http://www.swedishbiogas.com/index.php/sv/referensanlaeggnigar/sverige/jordberga> (accessed February 6, 2015).
- [12] J. Murphy, R. Braun, P. Weiland, A. Wellinger, Biogas from crop digestion, IEA Bioenergy Task. (2011). <http://groengas.nl/wp-content/uploads/2013/07/2011-09-00-Biogas-from-Crop-Digestion.pdf>.
- [13] Wikipedia, Biogas, (2015). <http://en.wikipedia.org/wiki/Biogas> (accessed May 21, 2015).
- [14] A. Petersson, A. Wellinger, Biogas upgrading technologies—developments and innovations, IEA Bioenergy. (2009) 20. <http://typo3.dena.de/fileadmin/biogas/Downloads/Studien/IEA-BiogasUpgradingTechnologies2009.pdf>.

- [15] B. Molinuevo-Salces, R. Fernández-Varela, H. Uellendahl, Key factors influencing the potential of catch crops for methane production, *Environ. Technol.* 35 (2014) 1685–1694. doi:10.1080/09593330.2014.880515.
- [16] J. Berntsen, J.E. Olesen, B.M. Petersen, E.M. Hansen, Long-term fate of nitrogen uptake in catch crops, *Eur. J. Agron.* 25 (2006) 383–390. doi:10.1016/j.eja.2006.07.006.
- [17] J. Constantin, B. Mary, F. Laurent, G. Aubrion, A. Fontaine, P. Kerveillant, et al., Effects of catch crops, no till and reduced nitrogen fertilization on nitrogen leaching and balance in three long-term experiments, *Agric. Ecosyst. Environ.* 135 (2010) 268–278. doi:10.1016/j.agee.2009.10.005.
- [18] K. Möller, W. Stinner, G. Leithold, Growth, composition, biological N₂ fixation and nutrient uptake of a leguminous cover crop mixture and the effect of their removal on field nitrogen balances and nitrate leaching risk, *Nutr. Cycl. Agroecosystems.* 82 (2008) 233–249. doi:10.1007/s10705-008-9182-2.
- [19] M. Gunnarsson, Gödslade eller ogödslade mellangrödor som biogassubstrat ?, (2014).
- [20] E. Kreuger, T. Prade, F. Escobar, S.E. Svensson, J.E. Englund, L. Björnsson, Anaerobic digestion of industrial hemp-Effect of harvest time on methane energy yield per hectare, *Biomass and Bioenergy.* 35 (2011) 893–900. doi:10.1016/j.biombioe.2010.11.005.
- [21] N. Niemetz, K. Kettl, Economic and Ecological Potential Assessment for Biogas Production Based on Intercrops, (2012).
- [22] M. Galbe, G. Zacchi, Pretreatment: The key to efficient utilization of lignocellulosic materials, *Biomass and Bioenergy.* 46 (2012) 70–78. doi:10.1016/j.biombioe.2012.03.026.
- [23] D. Fengel, G. Wegener, *Wood: Chemistry, Ultrastructure, Reactions*, Walter de Gruyter & Co., Berlin, 1984.
- [24] S.V. Chang, M.T. Holtzaple, Affecting Biomass Enzymatic Reactivity, *Appl. Biochem. Biotechnol.* 84 (2000).
- [25] M. Kihlman, O. Wallberg, L. Stigsson, U. Germgård, Dissolution of dissolving pulp in alkaline solvents after steam explosion pretreatments, *Holzforschung.* 65 (2011) 613–617. doi:10.1515/HF.2011.094.
- [26] B.C. Saha, Hemicellulose bioconversion, *J. Ind. Microbiol. Biotechnol.* 30 (2003) 279–291. doi:10.1007/s10295-003-0049-x.
- [27] E. Klimiuk, T. Pokój, W. Budzyński, B. Dubis, Theoretical and observed biogas production from plant biomass of different fibre contents, *Bioresour. Technol.* 101 (2010) 9527–9535. doi:10.1016/j.biortech.2010.06.130.
- [28] L. Laureano-Perez, F. Teymouri, H. Alizadeh, B.E. Dale, Understanding factors that limit enzymatic hydrolysis of biomass, *Appl. Biochem.* 124 (2005) 1081–1099. doi:10.1007/978-1-59259-991-2_91.

- [29] M.S. Finstein, S. The, Q. Review, N. Dec, Review Review by : Melvin S . Finstein
Published by : The University of Chicago Press, 64 (2015) 495–496.
- [30] Wikipedia, Biogas, (n.d.). <http://en.wikipedia.org/wiki/Biogas> (accessed February 5, 2015).
- [31] J.A. Mouljin, M. Makkee, A.E. Van Diepen, Chemical Process Technology, Second Edi, John Wiley & sons Ltd, 2013.
- [32] E. Kreuger, The Potential of Industrial Hemp (*Cannabis Sativa*) for Biogas Production, Lund University; Biotechnology, 2012.
- [33] L. Appels, J. Baeyens, J. Degrève, R. Dewil, Principles and potential of the anaerobic digestion of waste-activated sludge, *Prog. Energy Combust. Sci.* 34 (2008) 755–781. doi:10.1016/j.pecs.2008.06.002.
- [34] V. a. Vavilin, B. Fernandez, J. Palatsi, X. Flotats, Hydrolysis kinetics in anaerobic degradation of particulate organic material: An overview, *Waste Manag.* 28 (2008) 939–951. doi:10.1016/j.wasman.2007.03.028.
- [35] F. Raposo, M. a. De La Rubia, V. Fernández-Cegri, R. Borja, Anaerobic digestion of solid organic substrates in batch mode: An overview relating to methane yields and experimental procedures, *Renew. Sustain. Energy Rev.* 16 (2012) 861–877. doi:10.1016/j.rser.2011.09.008.
- [36] a. T.W.M. Hendriks, G. Zeeman, Pretreatments to enhance the digestibility of lignocellulosic biomass, *Bioresour. Technol.* 100 (2009) 10–18. doi:10.1016/j.biortech.2008.05.027.
- [37] U. Mais, A.R. Esteghlalian, J.N. Saddler, S.D. Mansfield, Enhancing the enzymatic hydrolysis of cellulosic materials using simultaneous ball milling., *Appl. Biochem. Biotechnol.* 98-100 (2002) 815–832. doi:10.1385/ABAB:98-100:1-9:815.
- [38] S. Lafitte-Trouqué, C.F. Forster, The use of ultrasound and gamma-irradiation as pre-treatments for the anaerobic digestion of waste activated sludge at mesophilic and thermophilic temperatures., *Bioresour. Technol.* 84 (2002) 113–118.
- [39] B. Park, J.H. Ahn, J. Kim, S. Hwang, Use of microwave pretreatment for enhanced anaerobiosis of secondary slugde, *Water Sci. Technol.* 50 (2004) 17–23.
- [40] M. Taniguchi, H. Suzuki, D. Watanabe, K. Sakai, K. Hoshino, T. Tanaka, Evaluation of pretreatment with *Pleurotus ostreatus* for enzymatic hydrolysis of rice straw., *J. Biosci. Bioeng.* 100 (2005) 637–643. doi:10.1263/jbb.100.637.
- [41] M. Kurakake, N. Ide, T. Komaki, Biological pretreatment with two bacterial strains for enzymatic hydrolysis of office paper, *Curr. Microbiol.* 54 (2007) 424–428. doi:10.1007/s00284-006-0568-6.

- [42] R. Boopathy, Anaerobic biotransformation of furfural to furfuryl alcohol by a methanogenic archaeobacterium, *Int. Biodeterior. Biodegrad.* 63 (2009) 1070–1072. doi:10.1016/j.ibiod.2009.09.011.
- [43] J.L. Jones, K.T. Semrau, Wood hydrolysis for ethanol production — previous experience and the economics of selected processes, *Biomass.* 5 (1984) 109–135. doi:10.1016/0144-4565(84)90052-0.
- [44] P.M. Bondesson, M. Galbe, G. Zacchi, Comparison of energy potentials from combined ethanol and methane production using steam-pretreated corn stover impregnated with acetic acid, *Biomass and Bioenergy.* 67 (2014) 413–424. doi:10.1016/j.biombioe.2014.05.025.
- [45] M. Linde, M. Galbe, G. Zacchi, Steam Pretreatment of Acid-Sprayed and Acid-Soaked Barley Straw for Production of Ethanol, in: *Appl. Biochem. Biotechnol.*, Vol.129–13th ed., Lund, 2006: pp. 546–562.
- [46] O. Bobleter, Hydrothermal degradation of polymers derived from plants, *Prog. Polym. Sci.* 19 (1994) 797–841. doi:10.1016/0079-6700(94)90033-7.
- [47] S. McIntosh, T. Vancov, Enhanced enzyme saccharification of Sorghum bicolor straw using dilute alkali pretreatment, *Bioresour. Technol.* 101 (2010) 6718–6727. doi:10.1016/j.biortech.2010.03.116.
- [48] R. a. Silverstein, Y. Chen, R.R. Sharma-Shivappa, M.D. Boyette, J. Osborne, A comparison of chemical pretreatment methods for improving saccharification of cotton stalks, *Bioresour. Technol.* 98 (2007) 3000–3011. doi:10.1016/j.biortech.2006.10.022.
- [49] Wikipedia, Sorghum × drummondii, (2014). http://en.wikipedia.org/wiki/Sorghum_%C3%97_drummondii (accessed June 9, 2015).
- [50] C. Sambusiti, E. Ficara, F. Malpei, J.P. Steyer, H. Carrère, Influence of alkaline pretreatment conditions on structural features and methane production from ensiled sorghum forage, *Chem. Eng. J.* 211-212 (2012) 488–492. doi:10.1016/j.cej.2012.09.103.
- [51] C. Sambusiti, E. Ficara, F. Malpei, J.P. Steyer, H. Carrère, Effect of sodium hydroxide pretreatment on physical, chemical characteristics and methane production of five varieties of sorghum, *Energy.* 55 (2013) 449–456. doi:10.1016/j.energy.2013.04.025.
- [52] W. Cao, C. Sun, R. Liu, R. Yin, X. Wu, Comparison of the effects of five pretreatment methods on enhancing the enzymatic digestibility and ethanol production from sweet sorghum bagasse, *Bioresour. Technol.* 111 (2012) 215–221. doi:10.1016/j.biortech.2012.02.034.
- [53] C. Sambusiti, E. Ficara, M. Rollini, M. Manzoni, F. Malpei, Sodium hydroxide pretreatment of ensiled sorghum forage and wheat straw to increase methane production, *Water Sci. Technol.* 66 (2012) 2447–2452. doi:10.2166/wst.2012.480.
- [54] S. Zabihi, R. Alinia, F. Esmaeilzadeh, J.F. Kalajahi, Pretreatment of wheat straw using steam, steam/acetic acid and steam/ethanol and its enzymatic hydrolysis for sugar

production, *Biosyst. Eng.* 105 (2010) 288–297. doi:10.1016/j.biosystemseng.2009.11.007.

- [55] P.L. McCarty, *Anaerobic Waste Treatment Fundamentals*, Public Work. 95 (1964) 107–112.
- [56] *Bioprocess Control, AMPTS II Operation and Maintenance Manual*, Network. (2014).
- [57] A. Sluiter, D. Hyman, C. Payne, J. Wolfe, Determination of Insoluble Solids in Pretreated Biomass Material Laboratory Analytical Procedure (LAP) Issue Date : 03 / 21 / 2008, (2008) 1–9.
- [58] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, Determination of Ash in Biomass Laboratory Analytical Procedure (LAP) Issue Date : 7 / 17 / 2005, (2008).
- [59] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, Determination of structural carbohydrates and lignin in biomass, *Lab. Anal.* 2011 (2008). <http://www.nrel.gov/biomass/pdfs/42618.pdf>.
- [60] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, Determination of Extractives in Biomass Laboratory Analytical Procedure (LAP) Issue Date : 7 / 17 / 2005, (2008).
- [61] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, Determination of Sugars , Byproducts , and Degradation Products in Liquid Fraction Process Samples Laboratory Analytical Procedure (LAP) Issue Date : 12 / 08 / 2006, (2008).
- [62] L. Björnsson, M. Murto, T.G. Jantsch, B. Mattiasson, Evaluation of new methods for the monitoring of alkalinity, dissolved hydrogen and the microbial community in anaerobic digestion, *Water Res.* 35 (2001) 2835. doi:10.1016/S0043-1354(00)00585-6.
- [63] Wikipedia, Kjeldahl method, (2015). http://en.wikipedia.org/wiki/Kjeldahl_method.
- [64] B. Hames, C. Scarlata, a S. Nrel, Determination of Protein Content in Biomass, L. 42625. (2008).
- [65] M.G. Porter, R.S. Murray, The volatility of components of grass silage on oven drying and the inter-relationship between dry-matter content estimated by different analytical methods, *Grass Forage Sci.* 56 (2001) 405–411. doi:10.1046/j.1365-2494.2001.00292.x.
- [66] W. Parawira, M. Murto, R. Zvauya, B. Mattiasson, Anaerobic batch digestion of solid potato waste alone and in combination with sugar beet leaves, *Renew. Energy.* 29 (2004) 1811–1823. doi:10.1016/j.renene.2004.02.005.
- [67] T.R. Society, *P. Transactions, R. Society, P. Sciences*, Downloaded from <http://rsta.royalsocietypublishing.org/> on May 27 , 2015, (n.d.).

- [68] H.L. Chum, D.K. Johnson, S.K. Black, R.P. Overend, Pretreatment-Catalyst effects and the combined severity parameter, *Appl. Biochem. Biotechnol.* 24-25 (1990) 1–14. doi:10.1007/BF02920229.
- [69] K.J. Moore, H.J.G. Jung, Lignin and fiber digestion, *J. Range Manag.* 54 (2001) 420–430. doi:10.2307/4003113.
- [70] T. Amon, B. Amon, V. Kryvoruchko, A. Machmüller, K. Hopfner-Sixt, V. Bodiroza, et al., Methane production through anaerobic digestion of various energy crops grown in sustainable crop rotations, *Bioresour. Technol.* 98 (2007) 3204–3212. doi:10.1016/j.biortech.2006.07.007.
- [71] P. Weiland, Production and energetic use of biogas from energy crops and wastes in Germany., *Appl. Biochem. Biotechnol.* 109 (2003) 263–274. doi:10.1385/ABAB:109:1-3:263.
- [72] B. Molinuevo-Salces, S.U. Larsen, B.K. Ahring, H. Uellendahl, Biogas production from catch crops: Evaluation of biomass yield and methane potential of catch crops in organic crop rotations, *Biomass and Bioenergy.* 59 (2013) 285–292. doi:10.1016/j.biombioe.2013.10.008.
- [73] S. Overud, T. Lennartsson, Skötsel och restaurering av betesmarker och slåtterängar (in Swedish). *Jordbruksverket Report 2004:11*, 2004. doi:1102-3007.
- [74] L. Björnsson, M. del Pilar Castillo, C. Gunnarsson, S.-E. Svensson, O. Wallberg, Förbehandling av lignocellulosarika råvaror vid biogasproduktion Nyckelaspekter vid jämförande utvärdering, (2014).
- [75] A. Bauer, P. Bösch, A. Friedl, T. Amon, Analysis of methane potentials of steam-exploded wheat straw and estimation of energy yields of combined ethanol and methane production, *J. Biotechnol.* 142 (2009) 50–55. doi:10.1016/j.jbiotec.2009.01.017.
- [76] E.S. Heidrich, T.P. Curtis, J. Dolfing, Determination of the internal chemical energy of wastewater, *Environ. Sci. Technol.* 45 (2011) 827–832. doi:10.1021/es103058w.
- [77] Sciencelab.com, Material Safety Data Sheet Butyric acid MSDS, (2000) 1–5.
- [78] T. Amon, B. Amon, V. Kryvoruchko, W. Zollitsch, K. Mayer, L. Gruber, Biogas production from maize and dairy cattle manure-Influence of biomass composition on the methane yield, *Agric. Ecosyst. Environ.* 118 (2007) 173–182. doi:10.1016/j.agee.2006.05.007.
- [79] M. Neureiter, J.T. Pereira, C.P. Lopez, H. Pichler, R. Kirchmayr, Effect of silage preparation on methane yields from whole crop maize silages, 4th Int. 89Symp. Anaerob. Dig. Solid Waste. (2005) 109–115.
- [80] E. Kreuger, I. Nges, L. Björnsson, Ensiling of crops for biogas production: effects on methane yield and total solids determination, *Biotechnol. Biofuels.* 4 (2011) 44. doi:10.1186/1754-6834-4-44.

- [81] M. Linde, M. Galbe, G. Zacchi, Steam pretreatment of acid-sprayed and acid-soaked barley straw for production of ethanol., *Appl. Biochem. Biotechnol.* 129-132 (2006) 546–562. doi:10.1385/ABAB:130:1:546.
- [82] M. Galbe, G. Zacchi, Pretreatment of lignocellulosic materials for efficient bioethanol production, *Adv. Biochem. Eng. Biotechnol.* 108 (2007) 41–65. doi:10.1007/10_2007_070.
- [83] G. Antonopoulou, G. Lyberatos, Effect of pretreatment of sweet sorghum biomass on methane generation, *Waste and Biomass Valorization.* 4 (2013) 583–591. doi:10.1007/s12649-012-9183-x.
- [84] R. Boopathy, Methanogenic transformation of methylfurfural compounds to furfural, *Appl. Environ. Microbiol.* 62 (1996) 3483–3485. http://en.wikipedia.org/wiki/Sorghum_x_drummondii.
- [85] C. Bonini, M. D’Auria, P. Di Maggio, R. Ferri, Characterization and degradation of lignin from steam explosion of pine and corn stalk of lignin: The role of superoxide ion and ozone, *Ind. Crops Prod.* 27 (2008) 182–188. doi:10.1016/j.indcrop.2007.07.023.
- [86] G. Antonopoulou, K. Stamatelatou, G. Lyberatos, Exploitation of rapeseed and sunflower residues for methane generation through anaerobic digestion: The effect of pretreatment, *Chem. Eng. Trans.* 20 (2010) 253–258. doi:10.3303/CET1020043.
- [87] L. Pereira Ramos, The chemistry involved in the steam treatment of lignocellulosic materials, *Quim. Nova.* 26 (2003) 863–871. doi:10.1590/S0100-40422003000600015.

Appendices

Appendices I

In Figure 14 the first results are shown for the intermediate crops, which are redone due to errors in the experimental work.

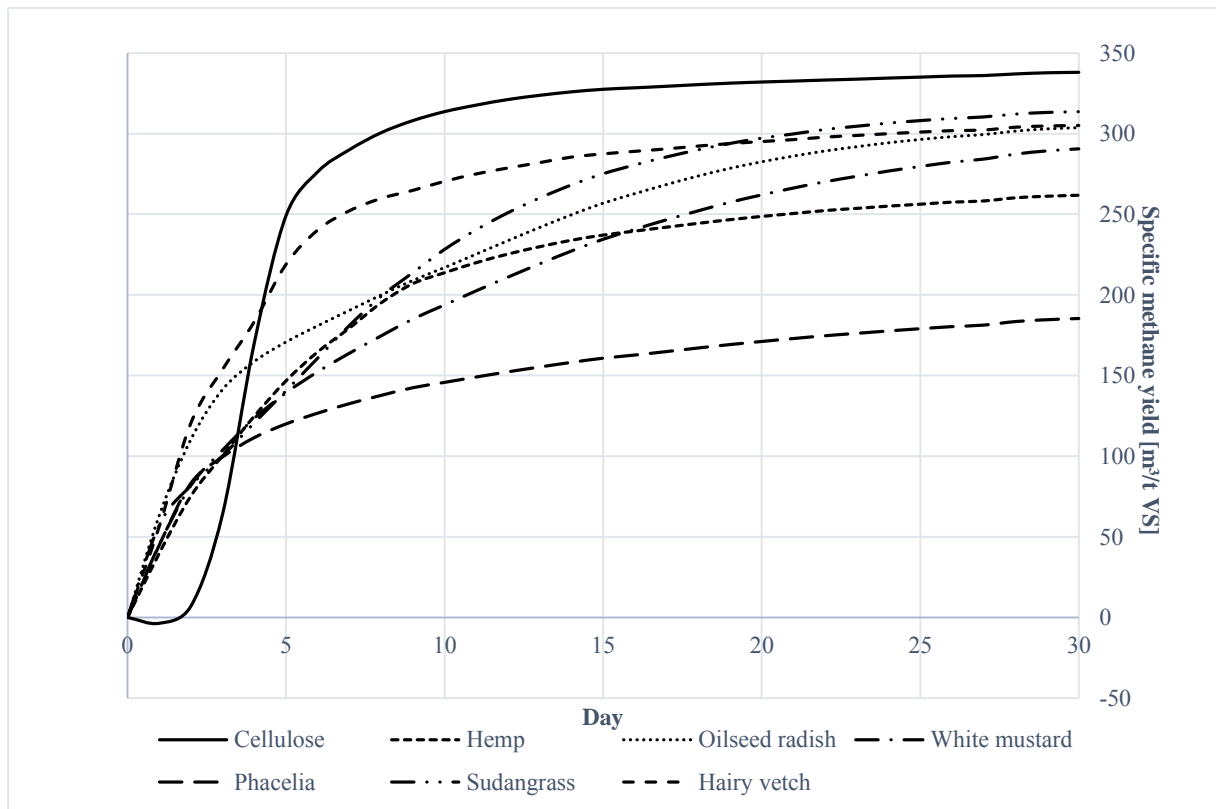


Figure 14. Accumulated specific methane yield during 30 days of batch digestion. Cellulose is included as a positive control.

Appendices II

The values are calculated as the example below with the molar mass and density given for acetic acid and methane in Table 10. In some cases the values were already calculated [74].

Example for acetic acid:

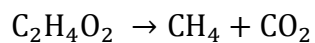


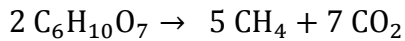
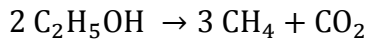
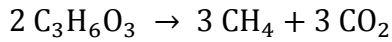
Table 10. Data of molar mass and density for acetic acid and methane.

Substrate	Molar mass (g/mol)	Density (g/dm ³)
Acetic acid	60.05	-
Methane	16.04	0.66

$$\text{Methane yield} = \frac{(M_{\text{CH}_4} \cdot n_{\text{CH}_4}) / (M_{\text{C}_2\text{H}_4\text{O}_2} \cdot n_{\text{C}_2\text{H}_4\text{O}_2})}{\delta_{\text{CH}_4}} = \frac{(16.04 \cdot 1) / (60.05 \cdot 1)}{0.66}$$

$$= 0.405 \text{ g/L}$$

The same procedure is repeated for lactic acid, ethanol and galacturonic acid. See equations below for reaction reactions.



Appendices III

Table 11 shows the biomass yield for the different intermediate crops.

Table 11. Shows the biomass yield for different intermediate crops.

Material	(kg TS/ha)
Phacelia	4973
White mustard	3235
Sudangrass	3493
Hemp	3673
Oilseed radish	4530
Hairy vetch	2000

Appendices IV

The pH on the ensiled crops can be seen in the Table 12.

Table 12. Measured pH of ensiled intermediate crops. Silage bucket number in parenthesis.

Material	Ensilage	pH
Phacelia	20 L (nr 8)	5.45
Phacelia	20 L (nr 8)	5.35
Phacelia	100 L	6.02
Phacelia	100 L	6.07
Phacelia	1000 L	8.04
Phacelia	1000 L	8.32
Sudangrass	1000 L	4.74
Sudangrass	1000 L	4.74
Hemp	20 L (nr 10)	5.4
Hemp	20 L (nr 10)	5.35

Appendices V

Figure 15 and 16 represent flowsheets of the intermediate crops respectively the steam pretreatment.

Intermediate crops

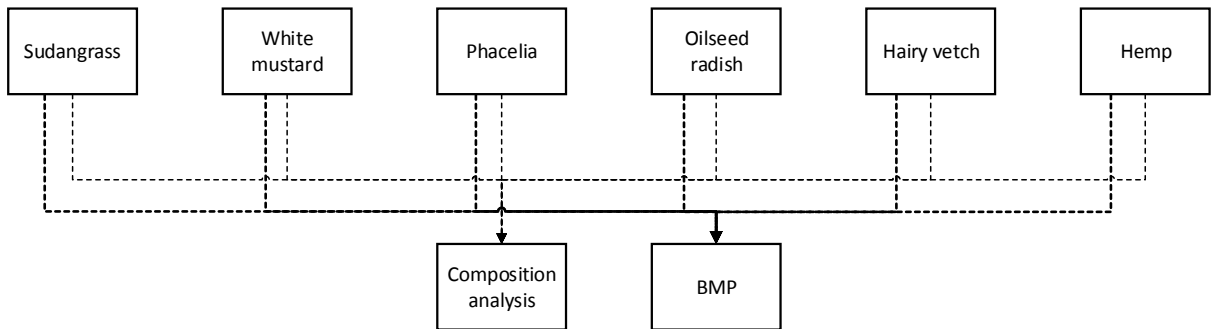


Figure 15. Flow sheet of analysis made of the intermediate crops.

Steam pretreatment

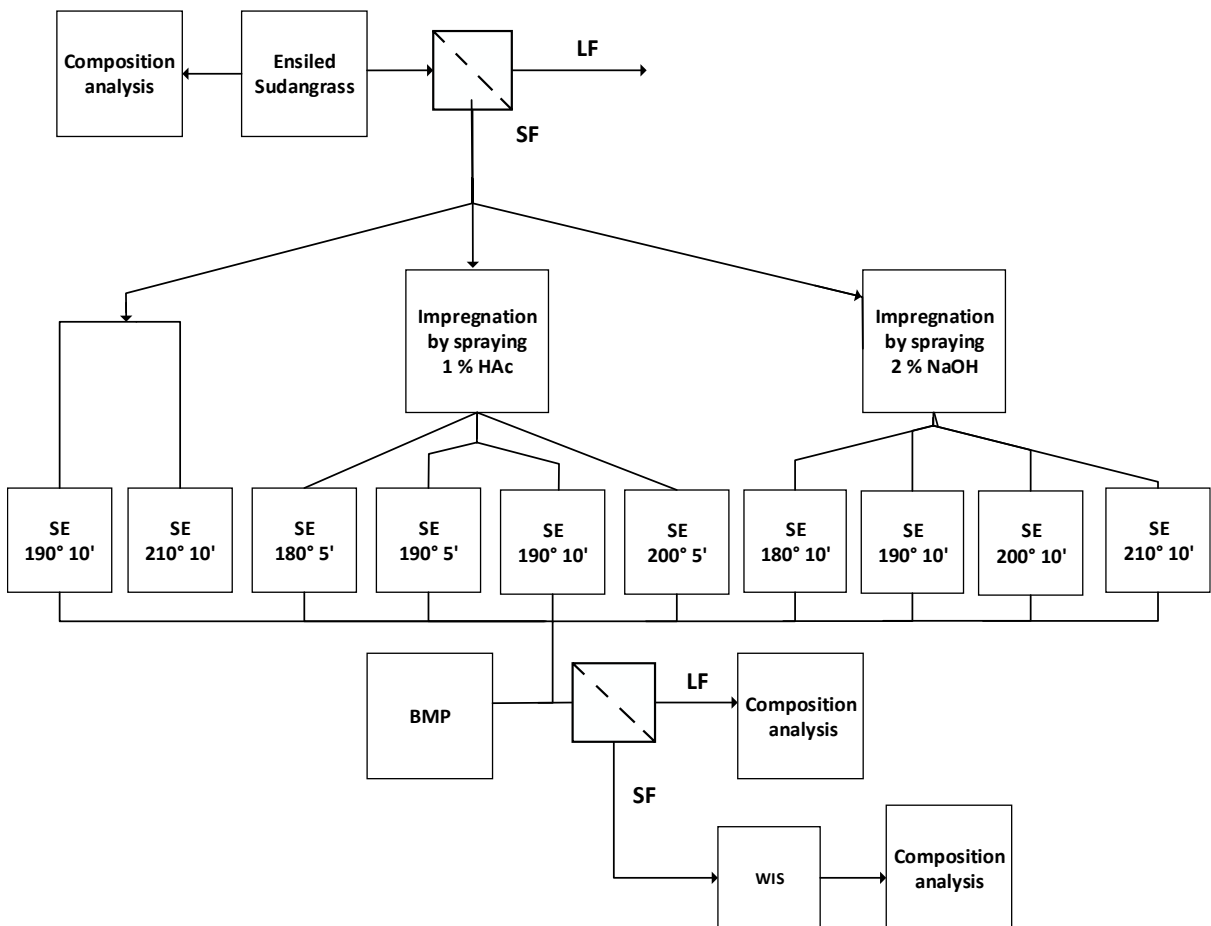


Figure 16. Flowsheet of the pretreatment done on ensiled sudangrass.

Appendices VI

Figure 17, 18 and 19 represent the mass balance from the different pretreatment conditions.

Steam pretreatment without catalyst

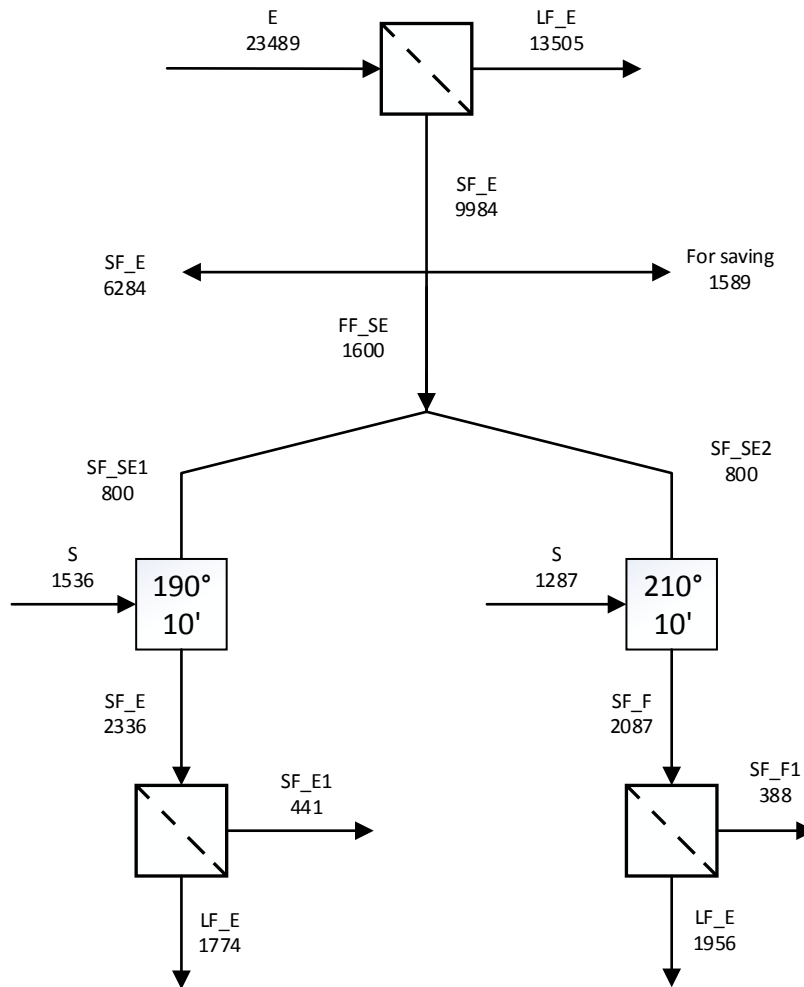


Figure 17. Specific flowsheet of the steam pretreatment of ensiled sudangrass without catalyst. All data are presented in gram.

Acetic acid

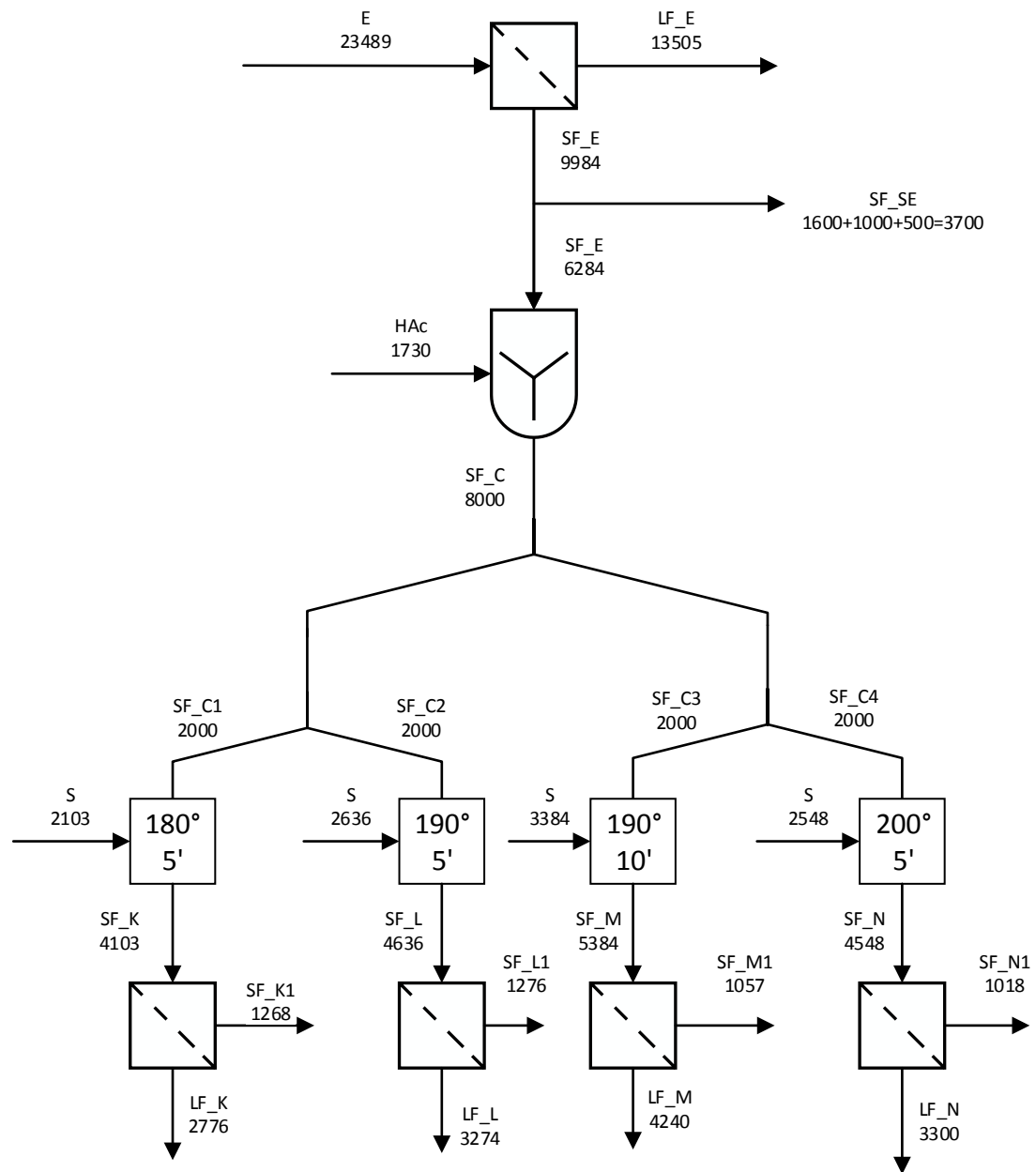


Figure 18. Specific flowsheet of the steam pretreatment of ensiled sudangrass without acetic acid catalyst. All data are presented in gram.

NaOH

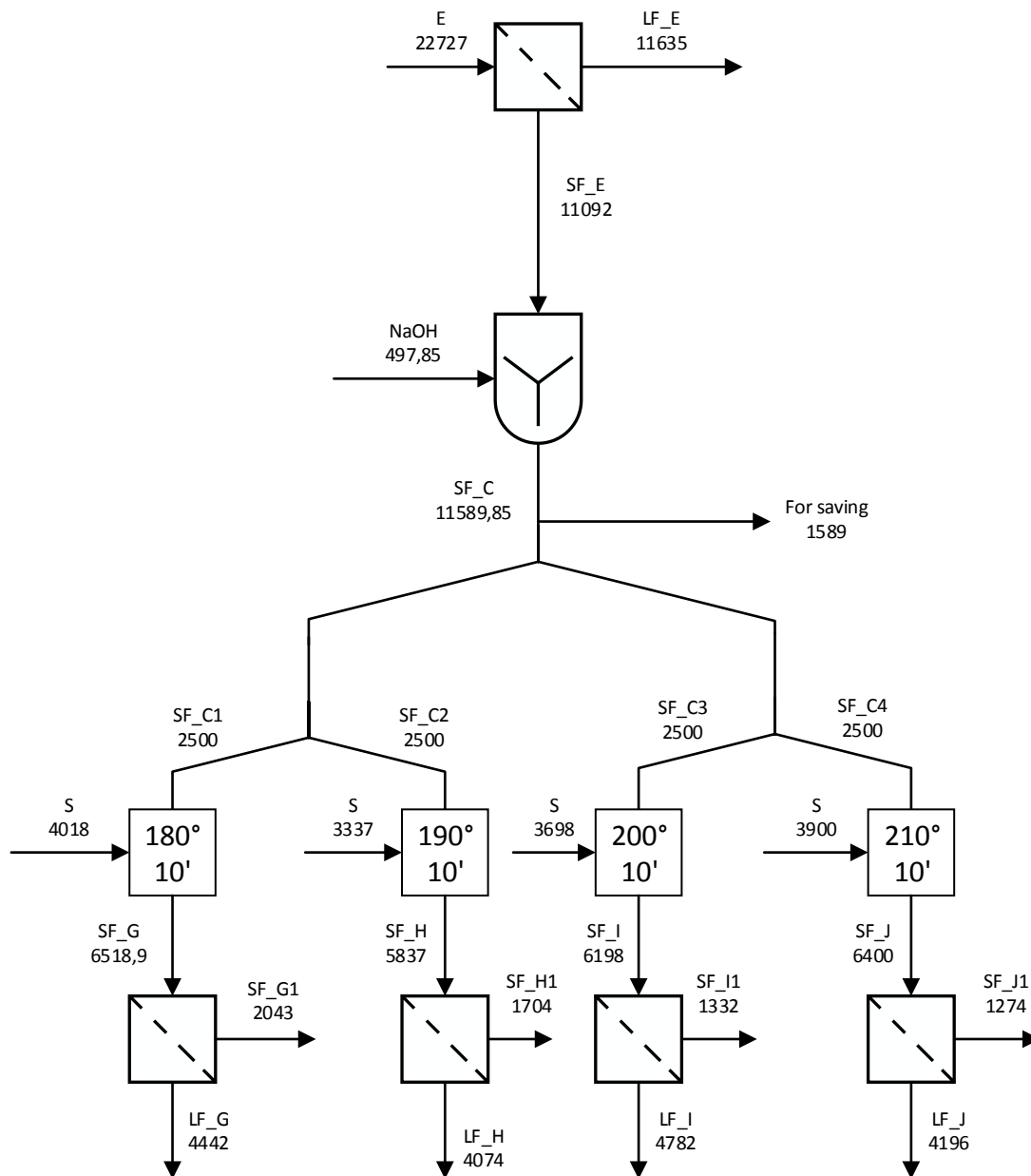


Figure 19. Specific flowsheet of the steam pretreatment of ensiled sudangrass without sodium hydroxide catalyst. All data are presented in gram.

Appendices VII

The tables 13-23 below represent data for SE of ensiled sudangrass, both for SE without catalyst and steam pretreatment with HAc and NaOH catalyst, g per 1 kg TS ensiled sudangrass.

Table 13. Results for the ensiled sudangrass. The results are presented in g/kg TS.

Ensiled sudangrass	WIS polymers	Liquid monomers	Liquid oligomers	Sum
Glucose	185.2	0.0	0.8	186.1
Xylose	134.8	0.1	0.4	135.3
Galactose	0.0	0.0	1.0	1.0
Arabinose	23.6	0.0	0.4	24.0
Mannose	1.5	n.d.*	0.1	1.6
Acid-soluble lignin	9.3	n.d.*	n.d.*	9.3
Acid-insoluble lignin	86.8	n.d.*	n.d.*	86.8
Lactic acid	n.d.*	n.d.*	19.2	19.2
Formic acid	5.8	n.d.*	n.d.*	5.8
Acetic acid	22.6	n.d.*	32.0	54.5
Levulinic acid	7.5	n.d.*	n.d.*	7.5
Ethanol	n.d.*	n.d.*	4.7	4.7
Galacturonic acid	n.d.*	0.2	2.9	3.1
Total				539.1

Table 14. Results for the steam pretreated sudangrass, condition SE 190 10. The results are presented in g/kg TS.

SE 190 10	WIS polymers	Liquid monomers	Liquid oligomers	Sum
Glucose	170.7	0.1	3.5	174.3
Xylose	56.5	0.5	29.4	86.3
Galactose	0.0	2.8	1.2	3.9
Arabinose	0.0	1.2	3.8	5.0
Mannose	1.0	0.5	1.1	2.6
Acid-soluble lignin	4.5	n.d.*	n.d.*	4.5
Acid-insoluble lignin	74.1	n.d.*	n.d.*	74.1
Lactic acid	n.d.*	n.d.*	2.7	2.7
Formic acid	3.9	n.d.*	n.d.*	3.9
Acetic acid	9.1	19.0	1.4	29.5
Levulinic acid	6.8	n.d.*	n.d.*	6.8
Ethanol	n.d.*	n.d.*	1.6	1.6
Galacturonic acid	n.d.*	0.9	0.2	1.0
Furfural	0.3	0.2	0.4	1.0
Total				397.2

Table 15. Results for the steam pretreated sudangrass, condition SE 210 10. The results are presented in g/kg TS.

SE 210 10	WIS polymers	Liquid monomers	Liquid oligomers	Sum
Glucose	181.5	2.3	2.4	186.3
Xylose	7.5	6.6	17.7	31.8
Galactose	2.3	0.0	3.1	5.4
Arabinose	0.0	2.0	1.0	3.1
Mannose	1.2	1.5	0.6	3.3
Acid-soluble lignin	5.0	n.d.*	n.d.*	5.0
Acid-insoluble lignin	104.5	n.d.*	n.d.*	104.5
Lactic acid	n.d.*	n.d.*	9.4	9.4
Formic acid	2.1	n.d.*	n.d.*	2.1
Acetic acid	2.6	n.d.*	29.4	32.1
Levulinic acid	2.5	n.d.*	n.d.*	2.5
Ethanol	n.d.*	n.d.*	1.9	1.9
Galacturonic acid	n.d.*	n.d.*	1.0	1.0
Furfural	0.0	n.d.*	0.9	0.9
Total				389.2

Table 16. Results for the steam pretreated sudangrass, condition NaOH 180 10. The results are presented in g/kg TS.

NaOH 180 10	WIS polymers	Liquid monomers	Liquid oligomers	Sum
Glucose	143.3	0.2	2.5	146.0
Xylose	87.5	0.2	7.8	95.6
Galactose	0.0	0.2	2.6	2.8
Arabinose	13.8	0.9	3.2	17.8
Mannose	0.0	0.7	0.5	1.2
Acid-soluble lignin	2.3	n.d.*	n.d.*	2.3
Acid-insoluble lignin	66.2	n.d.*	n.d.*	66.2
Lactic acid	n.d.*	n.d.*	9.0	9.0
Formic acid	2.6	n.d.*	n.d.*	2.6
Acetic acid	10.6	n.d.*	21.8	32.4
Levulinic acid	2.5	n.d.*	n.d.*	2.5
Ethanol	n.d.*	n.d.*	1.4	1.4
Galacturonic acid	n.d.*	0.4	0.7	1.1
Furfural	n.d.*	0.0	0.1	0.2
Total				381.0

Table 17. Results for the steam pretreated sudangrass, condition NaOH 190 10. The results are presented in g/kg TS.

NaOH 190 10	WIS polymers	Liquid monomers	Liquid oligomers	Sum
Glucose	206.4	0.1	3.4	209.9
Xylose	110.2	0.2	17.2	127.6
Galactose	0.0	0.2	3.4	3.6
Arabinose	16.6	0.7	5.1	22.4
Mannose	0.0	0.7	0.7	1.4
Acid-soluble lignin	6.4	n.d.*	n.d.*	6.4
Acid-insoluble lignin	88.6	n.d.*	n.d.*	88.6
Lactic acid	n.d.*	8.4	1.8	10.2
Formic acid	3.9	n.d.*	n.d.*	3.9
Acetic acid	9.5	21.7	7.3	38.5
Levulinic acid	4.4	n.d.*	n.d.*	4.4
Ethanol	n.d.*	n.d.*	1.3	1.3
Galacturonic acid	n.d.*	0.5	0.7	1.2
Furfural	0.3	0.0	0.2	0.5
Total				519.8

Table 18. Results for the steam pretreated sudangrass, condition NaOH 200 10. The results are presented in g/kg TS.

NaOH 200 10	WIS polymers	Liquid monomers	Liquid oligomers	Sum
Glucose	192.4	0.0	3.9	196.4
Xylose	60.5	0.9	31.2	92.6
Galactose	0.0	0.3	1.5	1.8
Arabinose	2.0	1.1	5.0	8.1
Mannose	1.0	0.9	0.5	2.5
Acid-soluble lignin	5.8	n.d.*	n.d.*	5.8
Acid-insoluble lignin	102.9	n.d.*	n.d.*	102.9
Lactic acid	n.d.*	8.4	2.2	10.6
Formic acid	3.7	n.d.*	n.d.*	3.7
Acetic acid	4.2	24.7	7.9	36.8
Levulinic acid	6.6	n.d.*	n.d.*	6.6
Ethanol	n.d.*	n.d.*	1.7	1.7
Galacturonic acid	n.d.*	n.d.*	0.4	0.4
Furfural	0.3	0.1	0.3	0.7
Total				470.7

Table 19. Results for the steam pretreated sudangrass, condition NaOH 210 10. The results are presented in g/kg TS.

NaOH 210 10	WIS polymers	Liquid monomers	Liquid oligomers	Sum
Glucose	177.7	0.0	3.6	181.2
Xylose	19.4	1.6	25.6	46.6
Galactose	0.0	0.0	2.9	2.9
Arabinose	0.0	1.0	3.0	4.0
Mannose	0.0	0.9	0.7	1.6
Acid-soluble lignin	4.4	n.d.*	n.d.*	4.4
Acid-insoluble lignin	88.6	n.d.*	n.d.*	88.6
Lactic acid	n.d.*	8.2	1.7	9.9
Formic acid	2.1	n.d.*	n.d.*	2.1
Acetic acid	1.9	21.1	7.9	30.9
Levulinic acid	3.6	n.d.*	n.d.*	3.6
Ethanol	n.d.*	n.d.*	1.8	1.8
Galacturonic acid	n.d.*	0.6	0.2	0.8
Furfural	0.2	0.2	0.3	0.7
Total				379.3

Table 20. Results for the steam pretreated sudangrass, condition HAc 180 5. The results are presented in g/kg TS.

HAc 180 5	WIS polymers	Liquid monomers	Liquid oligomers	Sum
Glucose	189.9	0.5	1.8	192.2
Xylose	115.3	0.5	6.0	121.9
Galactose	0.0	0.3	2.1	2.4
Arabinose	13.3	2.1	1.9	17.3
Mannose	0.7	0.4	0.6	1.7
Acid-soluble lignin	7.9	n.d.*	n.d.*	7.9
Acid-insoluble lignin	112.6	n.d.*	n.d.*	112.6
Lactic acid	n.d.*	n.d.*	4.8	4.8
Formic acid	3.8	n.d.*	n.d.*	3.8
Acetic acid	19.2	12.4	0.3	31.9
Levulinic acid	5.1	n.d.*	n.d.*	5.1
Ethanol	n.d.*	n.d.*	0.7	0.7
Galacturonic acid	n.d.*	0.4	0.7	1.0
Furfural	0.7	0.0	0.1	0.9
Total				504.1

Table 21. Results for the steam pretreated sudangrass, condition HAc 190 5. The results are presented in g/kg TS.

HAc 190 5	WIS polymers	Liquid monomers	Liquid oligomers	Sum
Glucose	172.7	0.0	3.7	176.3
Xylose	87.1	0.1	16.8	104.1
Galactose	0.0	0.4	3.2	3.7
Arabinose	9.4	1.6	4.1	15.1
Mannose	0.8	0.9	0.7	2.4
Acid-soluble lignin	8.1	n.d.*	n.d.*	8.1
Acid-insoluble lignin	123.2	n.d.*	n.d.*	123.2
Lactic acid	n.d.*	5.5	0.6	6.2
Formic acid	6.6	n.d.*	n.d.*	6.6
Acetic acid	15.6	9.2	6.6	31.4
Levulinic acid	10.6	n.d.*	n.d.*	10.6
Ethanol	n.d.*	n.d.*	1.0	1.0
Galacturonic acid	n.d.*	0.5	0.7	1.2
Furfural	0.8	0.1	0.3	1.1
Total				490.8

Table 22. Results for the steam pretreated sudangrass, condition HAc 190 10. The results are presented in g/kg TS.

HAc 190 10	WIS polymers	Liquid monomers	Liquid oligomers	Sum
Glucose	153.4	0.0	4.4	157.8
Xylose	51.7	0.6	35.0	87.3
Galactose	0.0	1.2	3.4	4.6
Arabinose	2.9	1.6	5.1	9.6
Mannose	1.6	0.6	1.7	3.8
Acid-soluble lignin	4.6	n.d.*	n.d.*	4.6
Acid-insoluble lignin	84.8	n.d.*	n.d.*	84.8
Lactic acid	n.d.*	n.d.*	6.2	6.2
Formic acid	2.4	n.d.*	n.d.*	2.4
Acetic acid	7.7	n.d.*	21.3	29.0
Levulinic acid	3.7	n.d.*	n.d.*	3.7
Ethanol	n.d.*	n.d.*	1.5	1.5
Galacturonic acid	n.d.*	1.1	0.0	1.2
Furfural	0.2	0.3	0.4	0.9
Total				397.3

Table 23. Results for the steam pretreated sudangrass, condition HAc 200 5. The results are presented in g/kg TS.

HAc 200 5	WIS polymers	Liquid monomers	Liquid oligomers	Sum
Glucose	161.4	0.0	2.8	164.2
Xylose	58.2	0.5	21.9	80.6
Galactose	0.0	1.2	1.6	2.8
Arabinose	1.2	1.3	3.0	5.5
Mannose	0.6	0.5	0.9	1.9
Acid-soluble lignin	5.9	n.d.*	n.d.*	5.9
Acid-insoluble lignin	106.4	n.d.*	n.d.*	106.4
Lactic acid	n.d.*	n.d.*	4.1	4.1
Formic acid	3.4	n.d.*	n.d.*	3.4
Acetic acid	10.7	n.d.*	9.6	20.3
Levulinic acid	4.8	n.d.*	n.d.*	4.8
Ethanol	n.d.*	n.d.*	0.8	0.8
Galacturonic acid	n.d.*	n.d.*	0.6	0.6
Furfural	0.5	0.2	0.3	1.0
Total				402.3

Appendices VIII

Shows the results for the pretreated solid fraction of the ensiled sudangrass NaOH 180 °C 10 min) for validation. The three scenarios were (1) complete extraction, (2) WIS followed by ethanol extraction and (3) only WIS. The results can be seen in Table 24. Table 25 shows the sum of the water and ethanol extractives in the untreated sudangrass solid fraction and in the solid fraction of pretreatment NaOH 180 °C 10 min.

Table 24. Composition of structural carbohydrates, lignin and byproducts for the solid fraction of sudangrass silage (pretreatment NaOH 180 °C 10 min). Expressed as g per 1 kg TS sudangrass silage input to the steam pretreatment scheme.

	Complete extraction	WIS + Ethanol extraction	WIS
AIL	242.8	54.5	66.2
ASL	20.4	4.3	2.3
Glucose	564.7	124.1	143.3
Xylose	420.5	87.6	87.5
Galactose	0.0	0.0	0.0
Arabinose	38.3	6.7	13.8
Mannose	3.9	1.5	0.0
Lactic acid	6.4	0.8	3.0
Glycerol	0.8	0.2	0.6
Formic acid	11.6	2.7	2.6
Acetic acid	42.7	9.0	10.6
Levulinic acid	16.2	3.9	2.5
Ethanol	5.3	1.3	1.6
HMF	8.9	3.0	2.7
Furfural	61.0	13.3	0.2
Galacturonic acid	4.1	0.0	1.0
Glucuronic acid	4.3	1.4	1.7

Table 25. Shows the extractives as g per 1 kg TS sudangrass silage input to the steam pretreatment scheme. B is the untreated ensiled sudangrass and G is the solid fraction of sudangrass silage (pretreatment NaOH 180 °C 10 min).

Condition	Extractives
B	260.9
G	217.9

Appendices IX

Table 26 below shows the pH of steam pretreated materials liquid fraction.

Table 26. pH of steam pretreated materials liquid fraction.

Conditions	pH
SE 190 10	4.29
SE 210 10	3.87
NaOH 180 10	4.88
NaOH 190 10	5.04
NaOH 200 10	4.73
NaOH 210 10	4.5
HAc 180 5	4.34
HAc 190 5	4.21
HAc 190 10	4.11
HAc 200 5	4.23