Lignocellulosic biorefinery with a thermo-biological pretreatment concept

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Declaration

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Witzenhausen, den 03.01.2023

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Preliminary remarks

This dissertation comprises papers that have already been published in international peer-reviewed journals and partly presented at conferences. The list referring to the chapter numbers in the thesis are provided below.

Chapter 3:
Comparative Life-Cycle-Assessment of pretreatment processes for the production of biofuels from lignocellulosic residues, P. Beuel, J. Bursche, C. Rieker, IEEE Xplore, 2019. DOI: 10.1109/IESC47067.2019.8976852

Chapter 4:
Biogenic catalysis by adding compost when using wheat straw in a biorefinery concept, P. Beuel, J. Bursche, C. Rieker, Chemical Engineering & Technology, Volume 43, Issue 8, 2020. DOI: 10.1002/ceat.202000029

Chapter 5:
Effects of thermo-biological pretreatments on the combustion properties of wheat straw in a cascaded biorefinery concept, P. Beuel, F. Torres, C. Rieker, J. Bursche, O. Hensel, Fuel, Volume 332, Issue P1, 2023. DOI: 10.1016/j.fuel.2022.125836

Conferences and poster presentations

Abstracts submitted as part of this research have been presented at various international conferences:

- *Untersuchung verschiedener Vorbehandlungsmethoden von Weizenstroh für den optimierten Einsatz von Lignocellulose in der Biogasproduktion*, P. Beuel, P.P. Silva, J. Bursche, J. Krämer, F. Rögener, C. Rieker; Poster at BIO-refined X: New ways in the use of biogenic raw materials, Feb. 26-27 in Oberhausen, Germany, 2019.
- *Vergleichendes Life-Cycle-Assessment von Verfahrenskombinationen zur Aufschließung lignocellulosehaltiger Reststoffe im Labormaßstab*; Oral presentation at the 20th Young Scientists Conference, June 18-19 in Merseburg, Germany, 2019.
- *Biogene Katalyse durch Kompostzusatz beim Einsatz von Weizenstroh in einem Bioraffineriekonzept*; Oral presentation at the 8th Status Conference Energetic Biomass Utilization – Bioenergy the X-Factor, Sept. 17-18 in Leipzig, Germany, 2019.
- *LCA of selected pretreatment processes for lignocellulosic residues for the production of biofuels*; Oral presentation at the 8th International Energy and Sustainability Conference, Oct. 17-18 in Farmingdale, USA, 2019.
- *Synergetische Effekte durch Mischung von Weizenstroh mit Apfelsaftnebenprodukten und Grüngutkompost in Biokonversionsprozessen*, P. Beuel, C. Rieker, S. Barbe, O. Hensel, J. Bursche; Poster at the 10th Status Conference Energetic Biomass Utilization, Nov. 29-30 in Leipzig, Germany, 2021. In: Tens, V.; Thrän, D. (Hrsg.): Reader Energetic Biomass Utilization. DOI 10.48480/3z9p-cy88.

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1 Introduction

The sustainable supply of the population with food, energy, and raw materials without harming the environment is a central task of society. Since the middle of the 20th century, fossil raw materials (crude oil and natural gas) have been among the most important sources of energy and raw materials worldwide (Brehmer 2008). Crude oil has been the primary raw material of industrial societies since that time (Cherubini 2010). As an energy source and feedstock for many chemical industrial products, crude oil is currently still the dominant raw material in the global economy (Cherubini 2010; Jain et al. 2022; Julio et al. 2017). In Germany, around 106 million tons of fossil crude oil are consumed annually (VCI 2019). Of this amount, 85% are used for the transport and energy sectors, while the rest is processed in the chemical industry. According to preliminary calculations by the *Federal Office of Economics and Export Control* (BAFA), 7.1 million tons of crude oil were imported into the Federal Republic of Germany in March 2021. The average price for a ton of crude oil free German border in March was 717.16 euros (BAFA 2021). In addition to the fluctuations in crude oil prices with an average upward trend (for comparison, crude oil price in March 2015 was 380.78 euros), the number of supplier countries has decreased from 29 to 18 (BAFA 2021; 2015). Apart from the economic and geopolitical dependence on supplier countries, the use of fossil raw materials leads to the release of climate-relevant greenhouse gases, since the provision of primary energy sources (e.g., oil and gas) and their conversion into useful energy (electricity and heat) cause gas emissions (Isikgor and Becer 2015; Liu et al. 2010). Climate change in particular increases the need for research in the field of developing processes for the use of biomass as an alternative raw material feedstock in the energy industry as well as in the chemical industry (Sawatdeenarunat et al. 2015).

The efficient and sustainable use of biomass is therefore the main focus of the German government's *National Research Strategy BioEconomy 2030* and is complemented by the *Roadmap Biorefineries* guideline (BMBF 2014; BMELV 2014). This is an important issue not only in Germany: In the last decade several bioeconomy strategies have been developed both in Europe and worldwide, each defining their national and international visions and measures to achieve a bio-based economy. Several important common aspects emerge from the strategies according to Bezama et al. (2019): National and global food security, sustainable agricultural production, healthy and safe food production, the industrial use of renewable resources, and the further development of biomass-based energy sources. The bio-based economy is defined as "technological development that leads to a significant replacement of fossil raw materials with biomass in the production of pharmaceuticals, chemicals, materials, fuels, electricity, and heat" (Sanders et al. 2010). The utilization of biogenic residues that are by-products of agriculture, such as harvest residues, organic waste, liquid manure, and dung, plays a major role in this context. The sustainable and efficient use of fiber-rich residual materials (such as lignocellulosic biomasses) as substrates that supply materials and energy can lead to synergy effects that close (carbon) material cycles and simultaneously replace fossil raw materials and save CO_2.

Synergistic usage effects introduce the concepts of "coupled production systems" (e.g., biorefineries) and "cascade use", in which an improvement in the use efficiency of a resource is achieved by successively upcycling/recycling the same resource to produce additional products, and the life cycle ends with a final energy use (Höglmeier, Weber-Blaschke, and Richter 2017). Based on the core objective of the bioeconomy defined above and the described synergy effects in the use of biogenic residues, a biorefinery concept, taking into account economics and ecology, represents an alternative to mitigate climate change and reduce the consumption of fossil fuels (Cherubini 2010; Venkata Mohan et al. 2016; BMBF 2014; BMELV 2014). Wheat straw appears to be a promising source of (lignocellulosic) biomass for such applications, considering that global wheat production in 2021 was 780 million tons, with about 354 million tons available for bioproduct and bioenergy production (Ingrao et al. 2021; USDA Foreign Agricultural Service 2022).

Given the organic properties of straw, it is not possible to fully digest the available organic components in fermentative processes to produce energy (García-Cubero et al. 2009). Direct use as a fuel is associated with technical difficulties due to the chemical composition (Ríos-Badrán et al. 2020). Suitable straw processing technology is therefore necessary for greater ecological and economic sustainability. For the use of this special substrate in a biorefinery, the above-mentioned properties must be optimized by suitable pretreatment processes (Ma, Shen, and Liu 2020; Kim and Dale 2004; Abraham et al. 2020; Das, Chanchal, and Roy 2015). Numerous pretreatment processes for energy and resource utilization from straw have been developed recently. Among these, biological pretreatment approaches have attracted increasing interest due to their cost efficiency and environmental friendliness (Ma, Shen, and Liu 2020).

Therefore, this thesis investigated whether the utilization and efficiency of wheat straw can be increased by a novel combination of processes incorporating a biological approach. The focus of this research was the cascade utilization of wheat straw in a combined process of biogas and solid fuel production. For this purpose, various process combinations were explored and modified to the extent that the conversion processes (anaerobic digestion and thermo-chemical utilization) were preceded by a combined pretreatment (hydrolysis by biological treatment with natural additives and thermal pressure hydrolysis as a sterilization step to ensure the effect of the additives) to increase efficiency, followed by an optimized separation of (lignocellulose) materials into a solid and a liquid phase. In addition to the technical development, an accompanying techno-ecological evaluation of the processes developed in the laboratory was also carried out and the transferability to large-scale applications was subsequently discussed.

1.1 Research question and research hypothesis

The overall objective of the thesis was the development of an eco-friendly pretreatment method for lignocellulosic biomass, whereby both the biodegradability and the combustion properties can be improved. The fundamental research question of this thesis, based on the process development carried out, was the following:

Is it possible to increase the degree of utilization and efficiency in the use of high-fiber residues such as wheat straw by a combined pretreatment process with a biological approach using natural additives while taking ecological aspects into account?

To answer the defined research question, the following hypotheses are evaluated using appropriate experimental approaches.

Hypothesis 1: Compared to chemical or physical-chemical pretreatment methods, the developed thermo-biological pretreatment is a resource-efficient, environmentally safe and low-energy process.

Experimental approach: Development of a Life-Cycle-Assessment (LCA) framework according to ISO 14040/14044 for biomass pretreatment methods for an accompanying ecological evaluation of the processes developed on a laboratory scale. Technical implementation of the LCA with software *GaBi*. Application of the LCA framework to selected pretreatment methods and determination of potential environmental impacts. Establishment of a methodology for comparative analysis of pretreatment processes for wheat straw lignocellulose according to ecological criteria. This hypothesis is reviewed in Chapter 3 and evaluated as well as further discussed in Chapter 6.

Hypothesis 2: The availability and quality of the lignocellulosic components cellulose, hemicellulose and lignin after thermo-biological pretreatment with green waste compost are comparable with established chemical or physical-chemical pretreatment methods.

Experimental approach: Determination of dry matter (DM) and volatile solids (VS) of lignocellulosic biomass according to the standard methods DIN EN 18134 and DIN EN 18122. To investigate the effects of pretreatments on lignocellulosic components using a high-performance liquid chromatograph (HPLC) to determine the chemical composition before and after applied pretreatment. Formation of a reference unit per kilogram DM respectively dimension (1 kg DM wheat straw) as a functional unit for the life cycle inventory to ensure comparability, since the fractionated lignocellulosic components differ both quantitatively and qualitatively after pretreatment (Li et al. 2021). Establishment of a methodology for comparative analysis of pretreatment processes for wheat straw lignocellulose according to technical criteria. This hypothesis is reviewed in Chapter 3 and Chapter 4 and evaluated as well as further discussed in Chapter 6.

Hypothesis 3: The preceding autoclaving causes thermo-hydrolytic decomposition, which favors a release of the contained sugars and at the same time influences the combustion properties by disintegration of the biomass matrix.

Experimental approach: Determination of DM and VS of lignocellulosic biomass according to the standard methods DIN EN 18134 and DIN EN 18122. To investigate the effects of autoclaving on the structure of lignocellulose using a HPLC to determine the chemical composition before and after applied pretreatment. Development of a workflow scheme for the analysis of combustion properties (such as calorific value according to ISO 18125, ash content according to ISO 18122, and ash melting behavior according to ISO 21404) of pretreated wheat straw. The hypothesis is reviewed in Chapters 4 and 5 and further discussed in Chapter 6.

Hypothesis 4: The use of green waste compost as a pretreatment additive causes lignocellulose degradation and increases bioconversion (amount and shortened time) during anaerobic digestion processes.

Experimental approach: Determination of dry matter DM and VS of lignocellulosic biomass and compost according to the standard methods DIN EN 18134 and DIN EN 18122. To investigate the effects of green waste compost on lignocellulosic degradation using a HPLC to determine the chemical composition before and after applied pretreatment. Determination of the methane yield with batch tests according to VDI Guideline 4630 for further evaluation of bioconversion in relation to the effects of green waste compost as a pretreatment additive. The hypothesis is evaluated in Chapters 4 and 5 and further discussed in Chapter 6.

Hypothesis 5: The use of liquid digestate as a pretreatment additive causes lignocellulose degradation and increases bioconversion (amount and shortened time) during anaerobic digestion processes.

Experimental approach: Determination of dry matter DM and VS of lignocellulosic biomass and liquid digestate according to the standard methods DIN EN 18134 and DIN EN 18122. To investigate the effects of liquid digestate during pretreatment using a HPLC to determine the chemical composition before and after applied pretreatment. Determination of the methane yield with batch tests according to VDI Guideline 4630 for further evaluation of bioconversion in relation to the effects of liquid digestate as a pretreatment additive. This hypothesis is evaluated in Chapters 4 and 5 and further discussed in Chapter 6.

Hypothesis 6: There is an improvement of combustion properties due to the biological pretreatments, in addition to the increase of methane yield.

Experimental approach: Determination of dry matter DM and VS of lignocellulosic biomass and liquid digestate according to the standard methods DIN EN 18134 and DIN EN 18122. Evaluation of the effects of pretreatments on combustion characteristics (such as calorific value according to ISO 18125, ash content according to ISO 18122, and ash melting behavior according to ISO 21404) using an analysis of variance (ANOVA). Determination of the methane yield with batch tests according to VDI Guideline 4630 for further evaluation of the pretreatment carried out and comparison with equivalent processes. The hypothesis is evaluated in Chapters 4 and 5 and further discussed in Chapter 6.

1.2 Research methodology

The focus of this research project was the extensive conversion of the energetically relevant organic components of wheat straw in an experimental approach on a laboratory scale. To this purpose, after testing various comminution techniques and subsequent homogenization of the substrate, thermal pressure hydrolysis was tested to break down the lignocellulosic fraction, additional biological pretreatment was carried out, and the material streams were separated into a solid and a liquid phase by physical separation processes. A schematic representation of the process combination is shown in Figure 1.

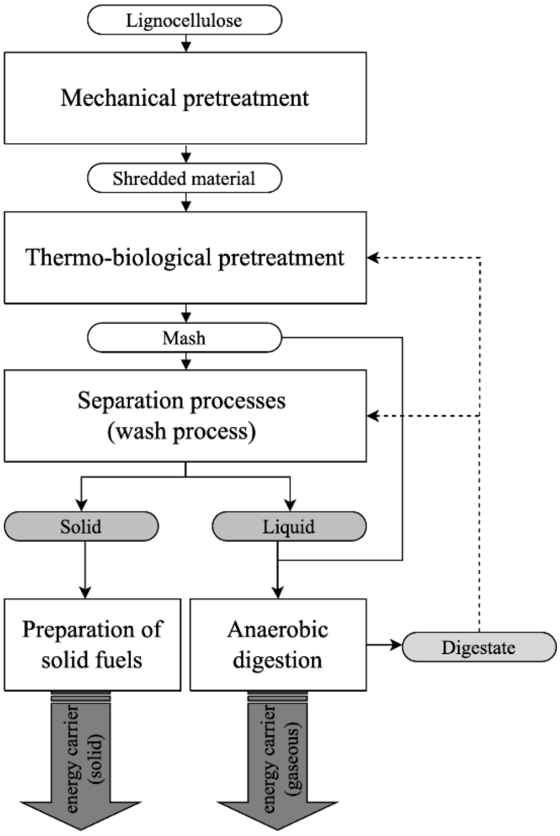

Figure 1: Process scheme adapted from VDI 6310.

It was expected that the liquid phase contains inorganic and easily fermentable substances and the solid phase is characterized by higher lignin contents. Based on these properties, the press juice was considered primarily for fermentation in biogas reactors and the press cake for the production of solid fuels, and their suitability for these conversion processes was examined.

Liquid digestate and green waste compost were used as additives for the biological treatment of biomass (degradation of lignocellulose). Sterilization of the wheat straw was therefore required prior to the addition of the additives in order to distinguish the effect of the additives from the effect of other microbes for research. An autoclave capable of operating at up to 4 bar was available for preconditioning (sterilization of the feedstock) by means of thermal pressure hydrolysis. The solid residues generated after the separation process were pelletized into specimens and then their combustion properties were investigated and evaluated. All intermediate or co-products were utilized with the complete use of all feedstock components. In addition to the energy use path, biomethane can be used as a substitute for natural gas, reducing the use of fossil fuels and supporting the desired shift from fossil-based to bio-based industries. There are different utilization routes for biomethane, e.g., for the production of methanol, dimethyl ether, ammonia as fuel or platform chemicals (Moghaddam, Ahlgren, and Nordberg 2016). Furthermore, it was expected that metabolites resulting from the process chain would also be suitable as feedstocks for the synthesis of further products of the chemical industry. The process combinations outlined as the basis of a biorefinery concept were analyzed according to the ISO 14040 and ISO 14044 standards with regard to their environmental aspects and effects and compared with selected reference processes. Since the purely ecological comparative analysis omits important factors for the evaluation of the process results, e.g., quality and quantity of the separated lignocellulosic fractions, a comparison matrix according to VDI 2225 was developed for this purpose, which was merged separately with the LCA results and considered additional factors for the technical process evaluation.

1.3 Thesis structure

This dissertation consists of eight Chapters, which include some published manuscripts and is organized as shown in Table 1.

Table 1: Thesis outline and chapters description.

	CHAPTER	CONTENT
1	Introduction	Background, scope and research question with specific hypotheses of this study.
2	State of the art	Literature review on the challenges and the strategies relevant to the topic of this thesis.
3	Research article	Selection of biomass pretreatment methods specializing in lignocellulose (wheat straw), including the development and application of a LCA framework according to ISO 14040/14044 along with a comparison matrix according to VDI 2225 for the techno-ecological process evaluation.
4	Research article	Development and application of a pretreatment concept using green waste compost as a natural source of microorganisms for the degradation of lignocellulosic wheat straw after thermal treatment in an autoclave to improve bioconversion.
5	Research article	Investigation of the combustion properties (ash and moisture content, calorific value and ash melting behavior) of wheat straw after thermo-biological pretreatment and verification of the suitability of compost as an effective additive in the production of solid fuels.
6	General results and discussion	Techno-ecological comparison of the developed pretreatment concept with subsequent discussion of its transferability to large-scale applications, discussion of the observed results and critical review of the approach with regard to the research question as well as the hypotheses.
7	Conclusion	Concluding remarks, limitations, recommendations and approaches for further research.
8	Summary	Summary of the main results observed in this thesis.

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2 State of the art

In this thesis, the conversion of wheat straw for a lignocellulosic biorefinery concept was investigated. In addition to the reasons for the selection, the following chapter describes the theoretical background on the structural composition of lignocellulose, the potential of lignocellulose from straw, and the lignocellulosic biorefinery concept. It also presents and evaluates the state of the art in the field of pretreatment processes for lignocellulose. At the end, the implications from the state of the art for the developed pretreatment concept within the scope of this thesis are described.

2.1 Lignocellulosic biomass

Lignocellulose is the main component of all plants as well as agricultural products, hence it is the most widespread biomass on earth (Smith 2019). The use of plant biomass as a material and fuel goes hand in hand with the history of mankind, as even the Neanderthal developed crude wooden tools and used firewood (Anukam and Berghel 2021; Smith 2019). Currently, biomass has tremendous potential to replace some of the fossil feedstocks and fuels because it is abundant, inexpensive, and a renewable resource (Anukam and Berghel 2021; Tian, Zhao, and Chen 2018). The development of alternative renewable raw materials is therefore becoming increasingly important for both the energy sector and the chemical industry, with agricultural residues (e.g. cereal straw) currently being the most important raw materials for Germany for reasons of qualitative and quantitative availability (BMELV 2014; Jain et al. 2022; Brosowski et al. 2019). In the study conducted by Brosowski et al. (2019), a monitoring system was developed to investigate which alternative renewable raw materials are available and to what extent, and what additional contribution can be expected from improved usage. For the case study Germany, the authors indicated that the share of renewable energy sources could be increased by up to 18% if the use of cereal straw, cattle slurry, solid cattle manure, and green waste can be increased. The authors conclude that significant amounts of fossil fuel for buses, locomotives, barges, or ocean-going vessels could be replaced if these feedstocks were made available as biomethane, bio-CNG, or bio-LNG in the transportation sector. Cereal straw was identified as the most important of 77 resources examined in this study because it has both a high theoretical and the highest mobilization potential. Therefore, straw lignocellulose is an ideal biomass for material and energy applications and is described below with regard to its structure and potential.

2.1.1 Composition and molecular structure of (cereal straw) lignocellulose

Lignocellulose is composed of the sugar polymers cellulose and hemicellulose and the aromatic polymer lignin (Carpita and Gibeaut 1993; Chen 2014). In the cell wall, cellulose provides tensile and flexural strength, while lignin is responsible for the compressive strength of the plant. Hemicellulose links cellulose and lignin and provides the flexibility of the biopolymer (Ghaemi, Abdullah, and Ariffin 2019; Chen 2014; Carpita and Gibeaut

1993). A schematic illustration of the spatial structure of lignocellulose is shown in Figure 2.

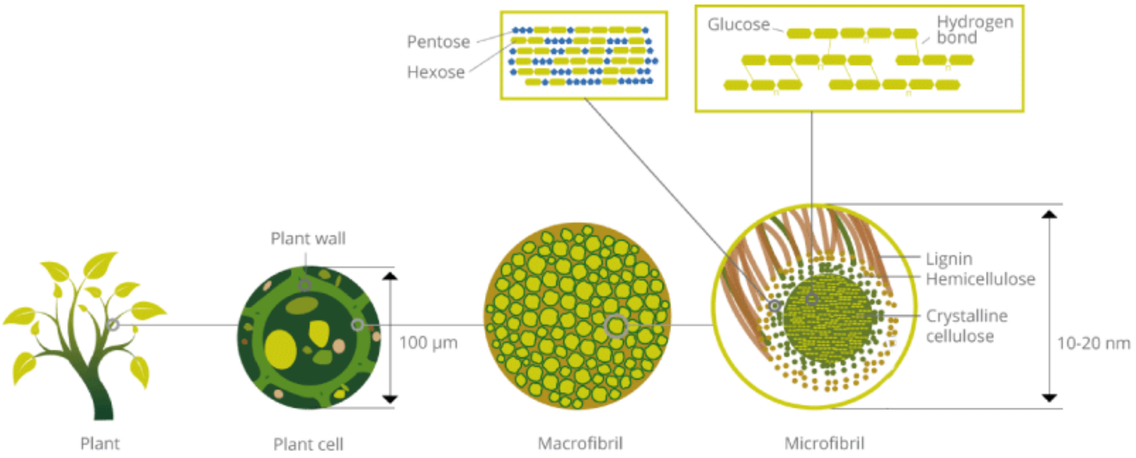

Figure 2: Schematic structure of lignocellulose adapted from Schulze (2018)

The structure of lignocellulose is well ordered. Plant cell walls are composed of macrofibrils with a diameter of 100-200 nm, which in turn are composed of several microfibrils (Ghaemi, Abdullah, and Ariffin 2019; Carpita and Gibeaut 1993; Ludwig 2014). The cell wall polysaccharides of lignocellulose consist of various monosaccharides bonded through a variety of chemical bonds (glycosidic bonds) (Schimpf 2014; Chen 2014; Carpita and Gibeaut 1993).

In the primary and secondary cell walls of wheat straw, cellulose is the predominant polysaccharide, with an average proportion of around 36% by mass (Schimpf 2014; Ghaemi, Abdullah, and Ariffin 2019; Tian, Zhao, and Chen 2018; Carpita and Gibeaut 1993; Palvasha et al. 2021). Cellulose is a biopolymer composed of β-D-glucose monomers (so-called C6-sugars) joined together by β-1,4- glycosidic bonds (position of the OH group at the C1 atom) to form a linear polymer (Anukam and Berghel 2021; Schimpf 2014). Due to the β-linkages in the macromolecules of cellulose, there is an alternating rotation of the glucose molecules of 180 degrees around the major axis of the cellulose molecule (Fabicovicova 2017). The 180-degree rotation leads to the formation of the disaccharide β-D-cellobiose, shown in Figure 3.

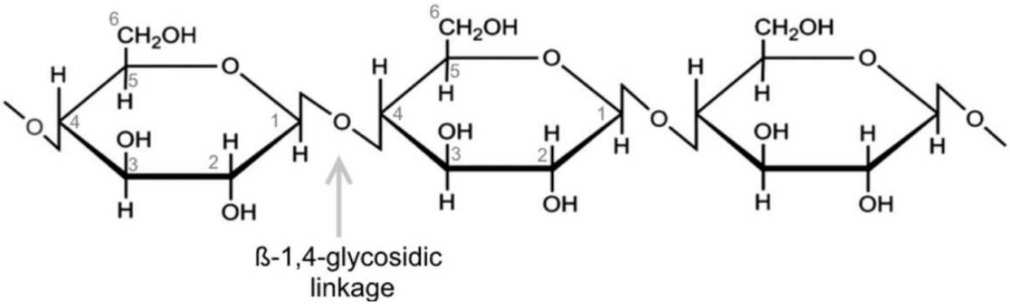

Figure 3: Cellulose structure adapted from Gurunathan, Mohanty, and Nayak (2015).

The repeat unit β-D-cellobiose is called the basic building block of cellulose, which consists of about 200 to 15,000 β-D-glucose units (Schimpf 2014; Gurunathan, Mohanty, and Nayak 2015; Cordeiro 2016). Due to the twisted arrangement of the glucose molecules in the structural unit cellobiose, two different ends of the molecular chains can be distinguished (Ludwig 2014; Chen 2014; Palvasha et al. 2021). The OH group of glucose bonded to the C1 carbon is referred to as the reducing end, since no further glucose monomer can be bonded here (Tofanica 2012; Fabicovicova 2017; Chen 2014). The second end of the glucose molecule at the C4-carbon is called the non-reducing end because of the possibility of glucose bonding due to a β-1,4-glycosidic linkage (Tofanica 2012; Fabicovicova 2017; Chen 2014; Betts et al. 1991). This arrangement provides an elongated structure of the cellulose chains and favors a parallel arrangement; the resulting linear cellulose fibers are additionally bound together by hydrogen bonds and the Van der Waals forces (interactions between atoms or molecules) (Tofanica 2012; Carpita and Gibeaut 1993; Chen 2014; Fabicovicova 2017; Schimpf 2014; Ludwig 2014). The high concentration of hydroxyl groups implies that cellulose tends to be hydrophilic by nature, although it is insoluble in water due to its compact and difficult-to-access structure (Cordeiro 2016).

In contrast to cellulose, hemicellulose is a heteropolymer composed of different monomers and is the second most abundant biopolymer in wheat straw, with an average proportion of around 25% by mass (Betts et al. 1991; Schimpf 2014; Fabicovicova 2017; Ludwig 2014). As shown in Figure 4, hemicellulose consists of the hexoses D-glucose, D-mannose, D-galactose, D-fructose, but mainly of the pentoses D-xylose and L-arabinose (so-called C5-sugars), which are arranged in different ways as backbone and side chains (Palvasha et al. 2021; Sun, Sun, and Tomkinson 2003; Ludwig 2014; Schimpf 2014).

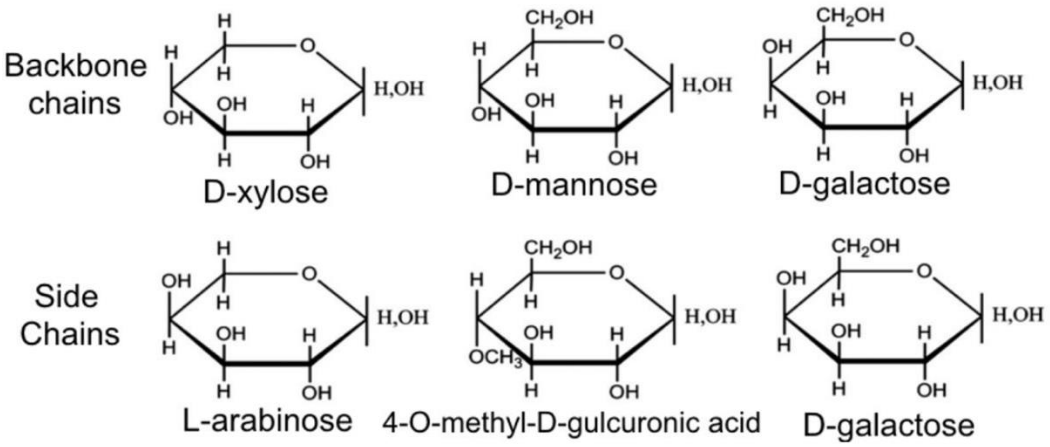

Figure 4: Various sugars in the macromolecules of hemicellulose, adapted from Gurunathan, Mohanty, and Nayak (2015).

Generally, the hemicellulose main chain is formed from one or two different monomers with lateral arrangement of the other sugars, resulting in a branched structure (Ludwig 2014; Schimpf 2014; Palvasha et al. 2021; Gurunathan, Mohanty, and Nayak 2015). Xylans represent the largest group among the hemicelluloses and consist mainly of the monomer D-xylose, but may also contain other monosaccharides (Fabicovicova 2017; Betts et al. 1991; Tofanica 2012; Tian, Zhao, and Chen 2018; Ludwig 2014; Schimpf 2014). Hemicelluloses form fewer hydrogen bonds than cellulose and have better solubility and lower rigidity (Ludwig 2014; Carpita and Gibeaut 1993; Tofanica 2012; Sun, Sun, and Tomkinson 2003). Along with lignin, hemicellulose acts as a kind of matrix for the cellulose microfibrils (Cordeiro 2016).

In addition to cellulose and hemicellulose, the cell wall of wheat straw is additionally permeated with lignin, which is referred to as "woodification" or "lignification"; lignin is the third most abundant component with an average mass proportion of about 23% (Smith 2019; Carpita and Gibeaut 1993; Ludwig 2014; Chen 2014). The lignin is firmly bonded to the cellulose respectively hemicellulose by ester bonds in the cell walls of the wheat straw (Palvasha et al. 2021; Ludwig 2014; Fabicovicova 2017; Schimpf 2014). Lignin is a highly branched hydrocarbon heteropolymer mainly formed by the polymerization of three cinnamyl alcohols, coniferyl, p-coumaryl and sinapyl alcohol (Cordeiro 2016; Chen 2014; Palvasha et al. 2021; Schulze 2018). Furthermore, the main lignin units are distinguished between the lignin types p-hydroxyphenyl (H), guaiacyl (G) and syringyl alcohol (S), which are subdivided into the lignin types G-lignins (mainly softwoods), GS-lignins (hardwoods) and HGS-lignins (e.g. wheat straw, miscantus) depending on the degree of lignification (Tofanica 2012; Schulze 2018; Ghaemi, Abdullah, and Ariffin 2019; Ludwig 2014). Figure 5 shows the structural composition of the lignin building block type HGS found in wheat straw.

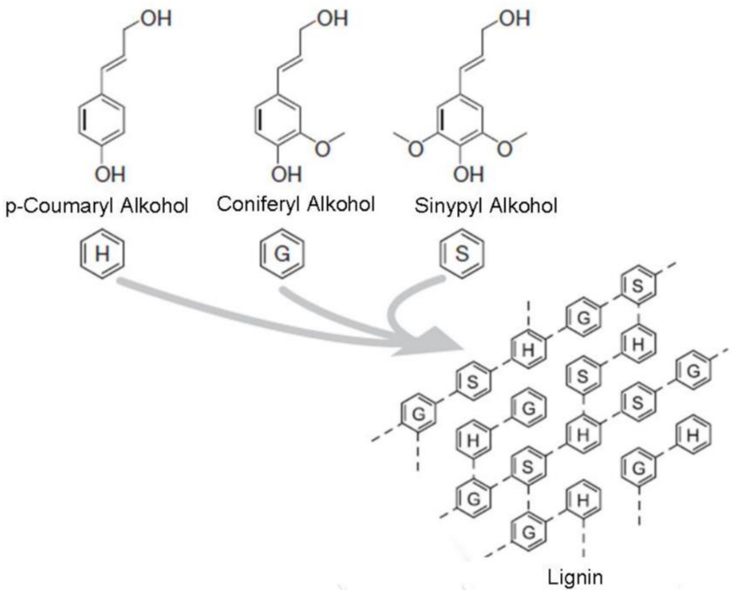

Figure 5: Structural composition of HGS lignin found in wheat straw; H: coumaryl, G: coniferyl, S: sinapyl alcohol, adapted from Rubin (2008).

As already mentioned, lignocellulose is the main component of the plant cell wall and consists of sugar and phenolic polymers. In order to obtain the polymers, the raw material must be fractionated, i.e., the raw material lignocellulose is separated into the main chemical components cellulose, hemicellulose and lignin in so-called biorefineries in order to produce a variety of bio-based products for material and energy use. Lignocellulose from straw has the greatest potential for technical applications. Therefore, the mobilization potentials and the market situation in Germany will be examined in more detail.

2.1.2 The utilization and market potential of cereal straw in Germany

Straw is the threshed and dried stalks of cereals, oil crops, fiber crops or legumes. It is a harvest residue and therefore classified as an agricultural residue (Zeller et al. 2012). Straw biomass is an important agricultural residue that can be used for the production of biogas, bioethanol, high-value biochemicals and biomaterials (Palvasha et al. 2021).

Based on the potential analysis of the *Deutsche Biomasseforschungszentrum* (DBFZ) from 2012 by Zeller et al., which is also referenced in the current edition of Kaltschmitt and Hartmann 2016, 215 million tons of agricultural residues are produced annually in Germany, including a theoretical potential of around 30 million tons of cereal straw. To determine cereal straw availability, yield and cropland data for 17 crop types and the corresponding grain-to-straw ratios were considered. Various humus balancing methods were used to determine the sustainable potential, ensuring that sufficient straw remained in the field for humus formation (Zeller et al. 2012). According to a more recent report by the DBFZ from 2019, studies on biomass potential agree that there is an annual straw potential of 33 to 38 million tons. Deducting the quantities needed, for example, to ensure soil quality (via humus balancing procedures), bedding, etc., between 5 and 13 million tons remain unused, which can be utilized for various alternative purposes (Pfeiffer et al.

2019). An article published in 2022 by the *Fachagentur Nachwachsende Rohstoffe e. V.* (FnR) states that the DBFZ estimates the unused straw potential in Germany at 4 to 9 million tons (Paul 2022). Assuming an average calorific value of 14.3 MJ kg^{-1} of cereal straw (Zeller et al. 2012), this results in an energy potential of 57 to 129 PJ.

To ensure a constant utilization of the straw throughout the year, the seasonal availability of straw must also be taken into account. Since cereal straw is produced when cereals are harvested, the growing seasons of the cereal types can be considered. Winter cereals are grown from September to November and harvested in July of the following year, while summer cereals are grown in March and also harvested in July (Zeller et al. 2012). The availability of wheat straw in Germany is very good compared to other cereals and was therefore selected as a lignocellulosic substrate for this research. Wheat can be harvested annually (July – August), is weather resistant and provides 6 to 14 tons of dry matter per hectare per year (winter as well as spring wheat) (Kaltschmitt and Hartmann 2016; Zeller et al. 2012). Hence, it can be concluded that the entire straw yield is produced in summer, so that its availability throughout the year depends on appropriate storage.

As Figure 6 illustrates, straw availability in Germany fluctuates between straw surplus and straw shortage. In some regions, there is theoretically more straw than is needed for agricultural purposes. The surplus regions are mainly located in the northeast of Germany, in central Germany around the Harz Mountains, and in some regions in northern Bavaria and Baden-Württemberg. Straw shortage regions probably cover their straw needs with straw from surrounding areas. The shortage regions are mainly located in the north and east of North Rhine-Westphalia and in parts of Lower Saxony and southern Germany.

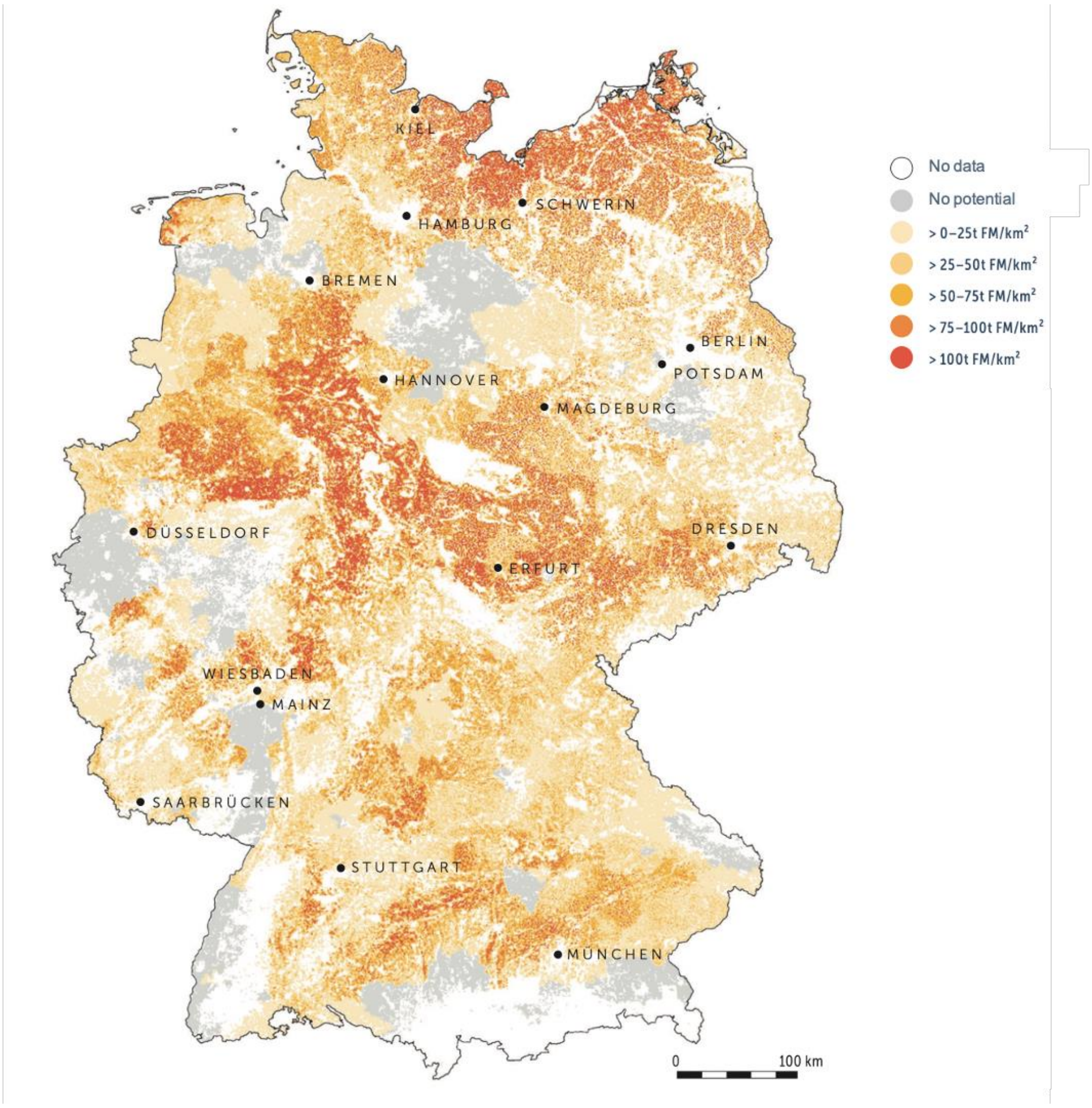

Figure 6: Straw potential at km² level in tons of fresh mass (FM) adapted from Pfeiffer et al. (2019).

In Germany, the straw market is regionally limited and characterized by fluctuating prices between 0 – 150 €/t DM or, e.g., 100 – 120 €/t DM for recreational horse demand (Pfeiffer et al. 2019). The straw price is determined by different benchmarks, e.g., the nutrient respectively humus value or on basis of the production costs of straw (Zeller et al. 2012; Pfeiffer et al. 2019; Brosowski et al. 2019). In summary, price fluctuations depend on the intended use, the associated straw cost, and the supply and demand of the resource. In comparison, silage corn varies between maize silage 70 to 170 €/t DM, prices for spruce or pine wood vary from 60 to 100 €/m^3 (Willert 2022; Neumann 2016).

Straw can be used in many ways and the technical options for material and energy use will continue to increase. Since the 1980s, straw or straw bales have been used as fuel in combined heat and power plants in Denmark (Voytenko and Peck 2012; Bentsen, Nilsson, and Larsen 2018). In order to make the use of straw in Germany more efficient, the DBFZ therefore proposes that Germany should establish an auction model comparable to that in Denmark (Voytenko and Peck 2012; Pfeiffer et al. 2019). Advanced technology concepts such as biorefineries are important drivers of resource demand, forcing the introduction of government support programs as well as monetary instruments (e.g. CO_2 taxes) to promote the use of bioenergy in general (Bentsen, Nilsson, and Larsen 2018).

2.2 Definition of biorefineries

A biorefinery is defined as an integrated technical concept that uses biomass as a multilayered raw material for the sustainable generation of various intermediate and end products such as materials, bioenergy and chemicals (Jain et al. 2022; Ragauskas et al. 2006; BMELV 2014; Kaltschmitt and Hartmann 2016; VDI 2016; Galbe and Wallberg 2019). The concept of a biorefinery is illustrated in Figure 7. The aim is to process all raw material components by means of different processes and technologies (Jain et al. 2022; Ragauskas et al. 2006; BMELV 2014).

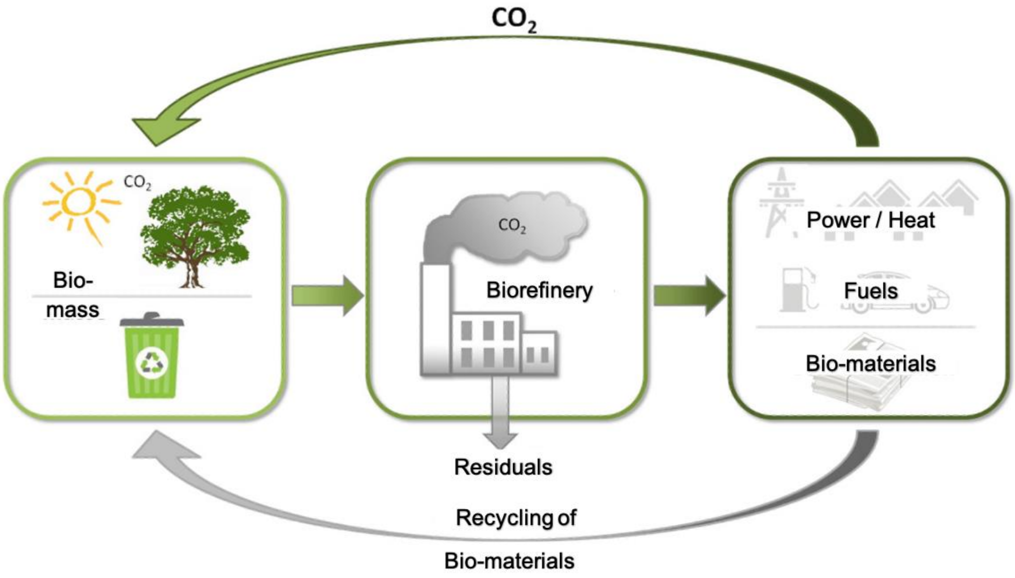

Figure 7: General concept of a biorefinery adapted from Ragauskas et al. (2006) and Fabicovicova (2017).

The processes along the entire value chain should be as closed as possible in terms of material and energy flows (Cristóbal et al. 2016; BMELV 2014). Both the use of materials and energy and the resource-conserving handling of auxiliary materials such as water and solvents must be taken into account in order to meet the requirements of sustainability and environmental protection. As part of this approach, a contribution can also be made to the development of the circular economy (Jain et al. 2022). As shown in Figure 8, the biorefinery process chain basically begins with the raw material, which is prepared with the use of a wide variety of plant components for pretreatment and conditioning of the biomass as well as for fractionation of the biomass components. The resulting platform can be processed into various energy and material products during subsequent conversion and downstream processing steps.

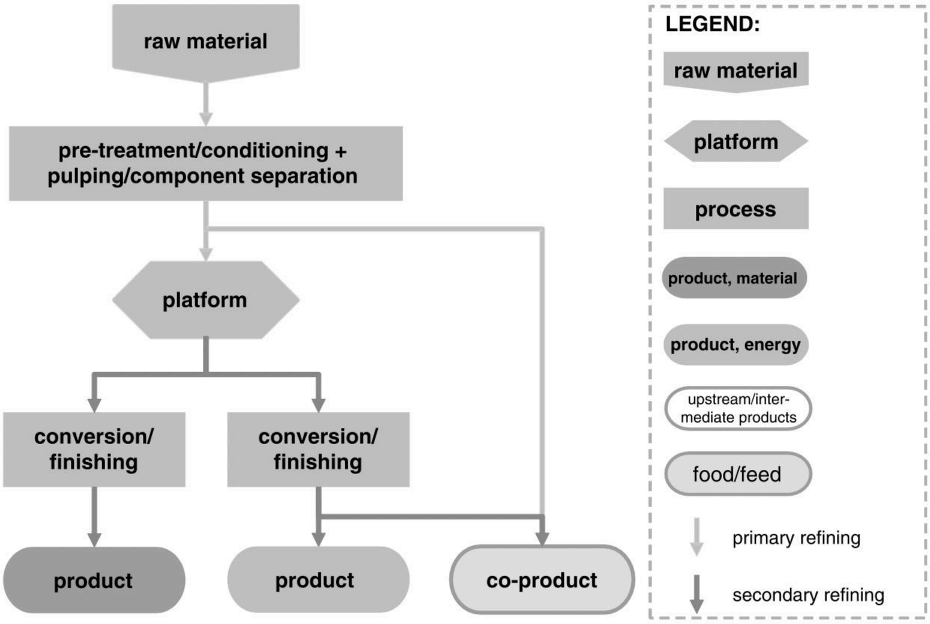

Figure 8: Classification system for biorefineries with the associated elements according to VDI 6310.

Accordingly, any biorefinery can be fundamentally divided into primary and secondary refinery process steps. Depending on the origin and processing of the biomass, different biorefinery concepts are distinguished. Essentially, five biorefinery concepts have proven to be particularly promising in research and development (BMELV 2014):

(1) sugar biorefinery or starch biorefinery

(2) vegetable oil biorefinery or algae lipid biorefinery

(3) synthesis gas biorefinery

(4) biogas biorefinery

(5) lignocellulosic biorefinery

According to a study of the Nova Institute by Carus (2017), a total of 224 biorefineries are distributed in Europe, which can be assigned to the above-mentioned categories. The highest number, at 64, are oil- and lipid-based biorefineries that produce biodiesel. Furthermore, five biorefineries are listed that process lignocellulose from other sources than wood. In Germany, there are only two industrial lignocellulosic biorefineries so far that do not use wood as feedstock – Süd-Chemie/Clariant in Straubing and Biowert in

Brensbach. Due to the straw potential described above, this thesis focuses on the concept of lignocellulosic biorefinery. In primary refining, the biomass components are fractionated into their main components (cellulose, lignin, hemicellulose). This is generally accomplished by pretreating and conditioning of the biomass (Figure 9). According to VDI 6310, these intermediate products (Lignin, C6- and C5-sugars) are also classified as platform chemicals, since they serve as feedstock for the secondary refinery and a variety of products can be manufactured from them. For economic reasons, component separation is usually carried out centrally at the biorefinery site, while pretreatment/conditioning can also be carried out decentralized in order to increase the feed radius of the biogenic raw materials (VDI 2016; da Silva, Torres Ortega, and Rong 2016).

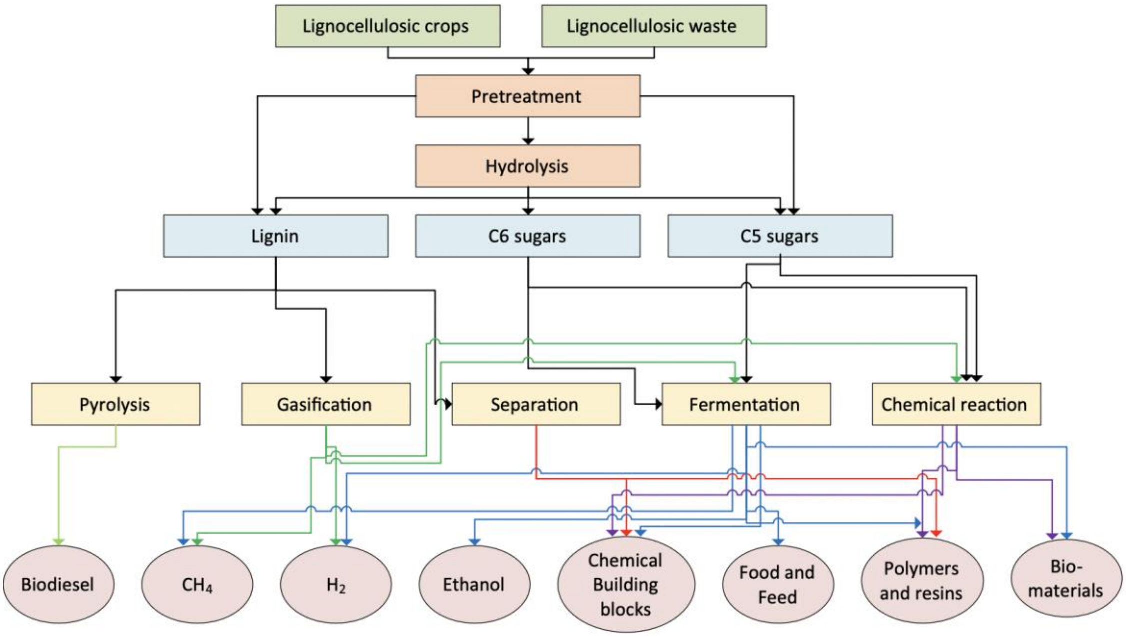

Figure 9: Schematic representation of a lignocellulosic biorefinery for the production of energy carriers and chemicals (Galbe and Wallberg 2019).

The different components can be separated and converted into a wide variety of products in secondary refining via further conversion and refining steps (Figure 8 and Figure 9). The co-products created in the biorefinery are further processed to provide process energy in the form of heat or electricity, or, if the legal requirements are met, to food or animal feed (Galbe and Wallberg 2019; Jain et al. 2022; Kumar and Sharma 2017; VDI 2016). This co-production is a characteristic feature of a biorefinery because, in contrast to conventional conversion, there is an internal recycling of the feedstocks that have not been fully converted (BMELV 2014; Jain et al. 2022).

According to Galbe and Wallberg (2019), the increasing interest in the use of lignocellulosic materials from agricultural, forestry, and other plants residues is mainly reflected in the number of publications in this field. Based on the literature database Scopus with the keywords "biomass" AND "pretreatment" it is apparent that from about 2010 onwards more than 1,000 publications per year were listed with the above keywords. In this context, the lignocellulose biorefinery concept is emerging as a promising alternative for many fossil-based products. The authors indicated that due to the wide variety of lignocellulosic materials, it is difficult to define a general process design for all lignocellulosic feedstocks. For example, the recalcitrance of softwood is much higher than that of most agricultural or herbaceous plants and forests. Hence, it is difficult to define the "most optimal" pretreatment method. Therefore, the following section describes various pretreatment methods and elaborates which pretreatment methods are best suited for the requirements of wheat straw.

2.3 Characterization of biomass pretreatment methods

For both biochemical and thermo-chemical conversion of biomass, pretreatment is a necessary process step intended to affect the recalcitrant nature of biomass through structural changes (Anukam and Berghel 2021; Ragauskas et al. 2006; A. K. Kumar and Sharma 2017; P. Kumar et al. 2009; Galbe and Wallberg 2019; Tian, Zhao, and Chen 2018). Therefore, pretreatment is one of the most important steps in a biorefinery, as it is the key to efficient biomass utilization by improving biomass properties (Galbe and Wallberg 2019; Anukam and Berghel 2021; Tan et al. 2021). Natural lignocellulosic biomass is basically resistant to direct enzymatic saccharification (Betts et al. 1991).

As described in section 2.1.1, this is due to the tight bonding of the polymer components cellulose (C6-sugars), hemicellulose (C5-sugars) and lignin in the cell walls of the biomass as well as the crystalline nature of cellulose. As shown in Figure 10, lignin binds hemicellulose and cellulose to form a physical barrier that provides an impermeable wall in the wheat straw biomass (Tian, Zhao, and Chen 2018; Carpita and Gibeaut 1993; Betts et al. 1991; Tan et al. 2021). With respect to a fermentative conversion of lignocellulose, Tian et al. (2018) note that the barriers, lignin and hemicellulose, result in a lower conversion of up to 20% of the original cellulose (which has not been converted or fragmented by any pretreatment method) into fermentable sugars. For this reason, disintegration of hemicellulose and lignin is essential prior to any bioconversion process to increase efficiency (see Figure 10).

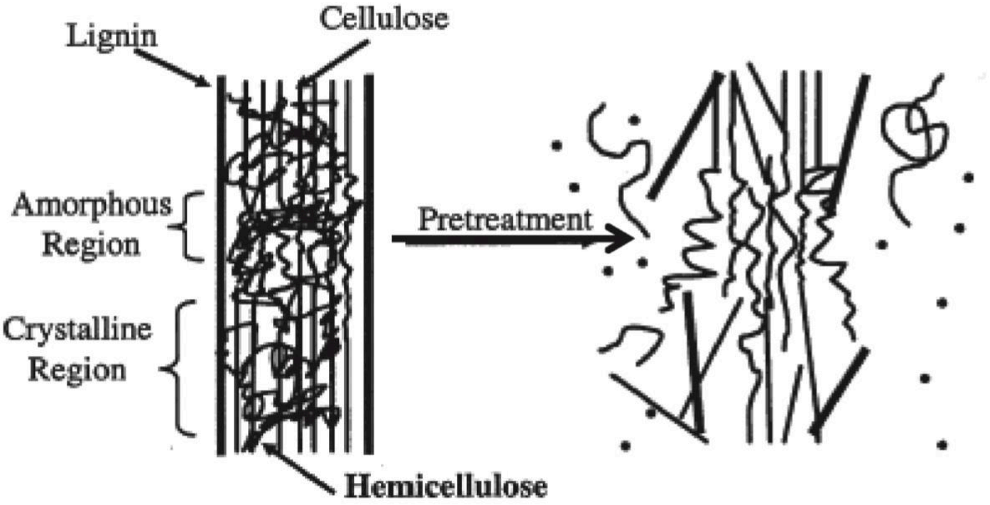

Figure 10: Effects of pretreatment on the surface and internal structure of lignocellulosic biomass, a schematic representation (Anukam and Berghel 2021).

However, today it is of great importance to find ways to maximize the overall yield of the valuable components of lignocellulosic materials (Galbe and Wallberg 2019). Pretreatment methods that allow efficient recovery of carbohydrates and lignin are consequently preferable. Furthermore, if enzymatic hydrolysis and fermentation steps are part of the overall process design, the formation of toxic or inhibitory compounds must be low to reduce the risk of adverse effects during bioconversion (Galbe and Wallberg 2019; Tian, Zhao, and Chen 2018; Anukam and Berghel 2021; Tan et al. 2021).

In recent years, various pretreatment methods have been investigated for the fractionation of a wide range of biogenic residues. Basically, four types of pretreatment methods are distinguished: physical-mechanical, physical-chemical, chemical and biological methods (see Figure 11).

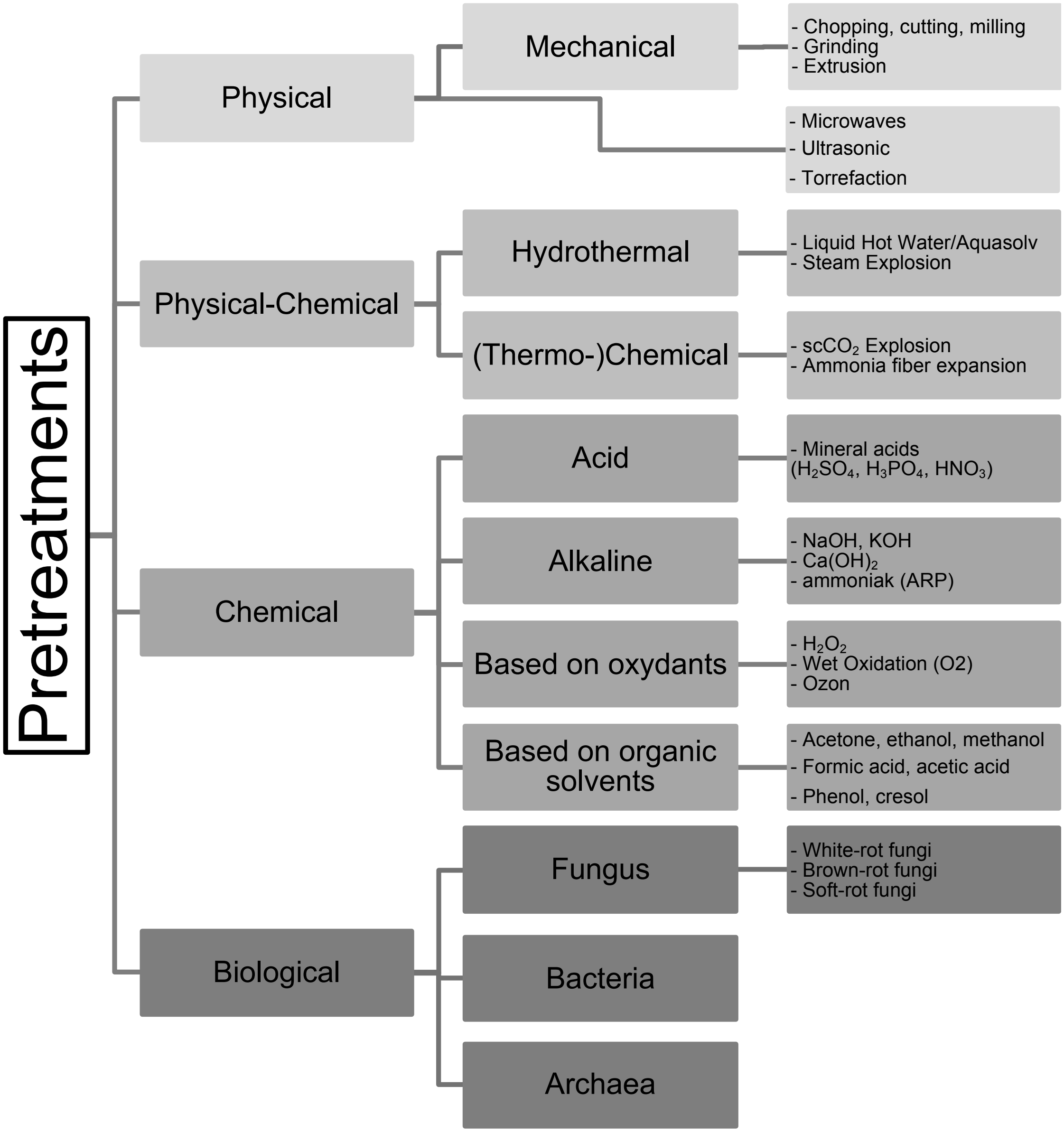

Figure 11: Classification of different pretreatment methods according to Tian, Zhao, and Chen (2018); P. Kumar et al. (2009); Galbe and Wallberg (2019); Palvasha et al. (2021); Anukam and Berghel (2021); VDI 6310 (2016); A. K. Kumar and Sharma (2017); Tan et al. (2021).

2.3.1 Physical pretreatment methods

Typically, physical processes use mechanical pretreatment methods such as chopping, shredding, cutting, milling and grinding. Mechanical pretreatment methods are often used as the first process step before all other pretreatment methods (Jiao et al. 2020; Smith 2019; Palvasha et al. 2021; Galbe and Wallberg 2019; A. K. Kumar and Sharma 2017; Anukam and Berghel 2021; Tan et al. 2021; Tian, Zhao, and Chen 2018; P. Kumar et al. 2009). The surface structure can be disrupted by mechanical pretreatment, resulting in a reduction of particle size in the bioreactor. Thus, the lignocellulose structure can be partially dissolved by lowering the degree of polymerization and crystallinity, while increasing the surface area, which favors the hydrolysis of cellulose by 5 – 25% (Anukam and Berghel 2021; Tian, Zhao, and Chen 2018; Galbe and Wallberg 2019; Tan et al. 2021).

Other physical pretreatment methods such as extrusion, microwaves and ultrasonic can also increase the areas of lignocellulosic biomass accessible to enzymes and microorganisms (Tan et al. 2021; Galbe and Wallberg 2019; Tian, Zhao, and Chen 2018; Anukam and Berghel 2021). Extrusion processing provides active sites that are easier to hydrolyze than non-pretreated material. This increases the hydrolysis of lignocellulose, as evidenced by higher glucose and xylose yields (Tian, Zhao, and Chen 2018). In Jiao et al. (2020), it was reported that by changing the particle size distribution and spatial structure through a twin-screw extrusion treatment of corn straw, an increased amount of glucose (45 g/L) and xylose (40 g/L) was obtained after enzyme digestion.

Microwave processing creates a mutual friction effect that raises the temperature of the raw material and causes a series of physical and chemical reactions such as heating and puffing (Tian, Zhao, and Chen 2018). The thermal energy generated by the collision of the molecules contributes to the expansion of the fibers and leads to the comminution of the biomass, which facilitates the subsequent hydrolysis efficiency (Tsegaye, Balomajumder, and Roy 2019). Ultrasonic treatment forms many small cavitation bubbles that cleave cellulose and hemicellulose fractions, resulting in an improvement of the hydrolysis process by increasing the accessibility of cellulose degrading enzymes (Tan et al. 2021; Galbe and Wallberg 2019; Tian, Zhao, and Chen 2018). Ultrasonic applications are commonly used as a complementary pretreatment process for lignocellulosic biomass, such as alkali treatment, which can improve bioconversion by disrupting the cell wall structure and increasing the specific surface area as well as reducing the degree of polymerization (Subhedar and Gogate 2016; A. K. Kumar and Sharma 2017; Dinh Vu et al. 2017).

Torrefaction is a mild pyrolysis process based on the thermal degradation of biomass at temperatures between 200 °C and 300 °C, mainly in an inert atmosphere (Ivanovski et al. 2022). In the study of Chiaramonti et al. (2011), the question of whether torrefaction is a suitable pretreatment process for the production of lignocellulosic second-generation bioethanol was investigated. Torrefaction appears to produce materials that can be enzymatically hydrolyzed and fermented to ethanol, with yields comparable to raw biomass, but much less energy efficient than steam explosion, for instance, due to the lower efficiency of hydrolysis and fermentation. However, torrefaction seems to be suitable for thermo-chemical conversion of lignocellulosic biomass, depending on the parameter setting such as temperature, residence time and heating rate, the energy density can be increased by 7 – 36% (Yan et al. 2009). The effects of temperature on the pyrolysis behavior and kinetics of cellulose during torrefaction were studied by Cao et al. The results showed that a solid product torrefied at the highest temperature had the greatest effect on oxygen removal and energy yield, as well as the highest carbon content and calorific value (Q. Wang et al. 2021). Torrefied biomass (biochar or black carbon) is a stable and carbon-rich solid bioproduct that can be used in thermo-chemical conversion processes and shows better performance in combustion, gasification and co-combustion (Ong et al. 2021).

2.3.2 Physical-chemical pretreatment methods

The physical-chemical methods usually use chemicals as catalysts or solvents and combine them with a change in pressure and temperature. For instance, the energy generated by the phase change of hot water is also used to defibrate biomass. The so-called "steam" explosion process was developed in 1926 and depressurizes hot water vapor in a saturated reactor chamber, causing the raw material (lignocellulose) to defiber due to the volume expansion of the vapor (D. Kumar and Murthy 2011; Stelte 2013). A liquid fraction is formed, containing primarily monomeric sugar building blocks from the decomposition of hemicellulose, and a solid fraction consisting of fermentable cellulose and the isolated lignin (Stelte 2013). The decomposition results are highly variable because they depend on residence time, particle size, temperature and pressure (D. Kumar and Murthy 2011; A. K. Kumar and Sharma 2017; P. Kumar et al. 2009; Kaltschmitt and Hartmann 2016). Steam explosion is performed at a temperature of 160 to 260 °C and a pressure of approx. 0.5 to 5 MPa for several seconds to minutes (Galbe and Wallberg 2019; Stelte 2013; Smith 2019; P. Kumar et al. 2009; Tian, Zhao, and Chen 2018; Tan et al. 2021; Palvasha et al. 2021).

The Liquid-Hot-Water (LHW) or Aquasolv process is a pretreatment method using liquid hot water at temperatures from 200 to 230 °C and high pressures up to 5 MPa (da Silva, Torres Ortega, and Rong 2016; Tan et al. 2021; Emeder 2021; Prasad et al. 2016; José A Pérez et al. 2007). As in Reynolds et al. (2016) reported, during treatment, the water shows acidic properties and acts as a catalyst for the hydrolysis of polysaccharides. Sulfur dioxide can also be used as an additional catalyst for further acidification (Tan et al. 2021). Based on the results obtained in Pérez et al. (2008), it can be concluded that in order to maximize the recovery of fermentable sugars in LHW pretreatment of wheat straw, the process must be carried out in two stages (hydrothermal process followed by enzymatic hydrolysis), as this allows the recovery of up to 80% of the xylose and up to 91% of the glucose content in the raw material.

Similar processes for physical-chemical pretreatment are (supercritical) carbon dioxide explosion ($scCO_2$) and ammonia fiber explosion (AFEX) at correspondingly moderate temperatures (Tan et al. 2021; D. Kumar and Murthy 2011; Galbe and Wallberg 2019). Instead of water, carbon dioxide (CO_2) or ammonia (NH_3) is used as the process liquid. In these processes, the lignocellulosic biomass is also fed into a reactor and additionally brought into contact with the corresponding process liquids (Galbe and Wallberg 2019).

In $scCO_2$ treatment of straw biomass, the CO_2 generates carbonic acid and increases the efficiency of enzymatic digestion (Tan et al. 2021). In addition, CO_2 can penetrate into the pores of hemicellulose, cellulose, and lignin, resulting in improved enzymatic hydrolysis and minimized inhibitors (Li et al. 2020). However, the high demand for reactor equipment limits its wide application (A. K. Kumar and Sharma 2017; Li et al. 2020).

In Lau et al. (2010), AFEX treatment was applied to various lignocellulosic materials such as wheat straw, rice straw, corn cob/straw, and sugarcane bagasse to reduce biomass recalcitrance and thus increase hydrolysis activities and conversion efficiency (up to 90% of the maximum total yield within 72 hours after enzymatic hydrolysis).

2.3.3 Chemical pretreatment methods

Chemical pretreatment involves the use of concentrated or dilute acids, alkalis, oxidizing agents or organic solvents such as alcohols, organic acids or aromatic compounds. Acid pretreatments can be used to selectively hydrolyze the glycosidic bonds of hemicellulose. However, the soluble sugars formed can further react during acid digestion to result in sugar degradation products such as furfural, 5-hydroxymethylfurfural (HMF), formic acid or levulinic acid (Tian, Zhao, and Chen 2018; Tan et al. 2021).

The alkaline pretreatments are characterized by low process temperatures (approx. 60 – 120 °C), but require a higher material input, since part of the alkali ions are converted into salts and bound in the biomass (Tan et al. 2021; Anukam and Berghel 2021; Tian, Zhao, and Chen 2018; Galbe and Wallberg 2019). With alkaline pretreatments, lignin can be degraded into its monomers and part of the hemicellulose into oligomers (Tian, Zhao, and Chen 2018; Raita et al. 2017; Dinh Vu et al. 2017). Alkali loading, reaction time and temperature are the most important effective factors for lignin removal and fermentable sugar production (D. Kumar and Murthy 2011; Raita et al. 2017; P. Kumar et al. 2009).

Oxidizing agents such as hydrogen peroxide, peroxyacetic acid, compressed oxygen, or ozone directly attack the lignin structure and can also dissolve hemicellulose and cellulose (Tan et al. 2021; Emeder 2021; P. Kumar et al. 2009). In most cases, however, oxidative processes also produce a number of toxic degradation products, especially soluble aromatics (Ludwig 2014; Emeder 2021; P. Kumar et al. 2009; Tan et al. 2021).

Other frequently studied pretreatment processes are the so-called Organo-Solv processes. Organo-Solv processes comprise a variety of methods that use either organic acids (acetic acid or formic acid), alcohols (methanol, ethanol or ketones like acetone) or aromatics such as phenol as solvents in combination with water (Tian, Zhao, and Chen 2018; Tan et al. 2021). Lignin and, in combination with temperature exposure (> 180 °C), hemicellulose can also be dissolved from the fiber by Organo-Solv treatment (Tan et al. 2021; Smith 2019; Tian, Zhao, and Chen 2018; P. Kumar et al. 2009; Galbe and Wallberg 2019; Raita et al. 2017; Zhao et al. 2017). The required process temperatures can be lowered by adding small amounts of a mineral acid (e.g., magnesium chloride and sulfite, calcium chloride and sodium hydroxide), since the H+ ions of the acid catalyze the decomposition of the glycosidic bonds as Zhao et al. (2017) reported. Particularly common solvents are lower alcohols such as ethanol or methanol due to their good delignification and utilization possibilities (Tian, Zhao, and Chen 2018; Tan et al. 2021; Galbe and Wallberg 2019; Anukam and Berghel 2021). The use of acetone is also suitable, as it exhibits better delignification than ethanol when processing wheat straw (acetone-water ratio:

50:50 w/w), which increases the lignin quality and the recoverability of the sugar fractions during enzymatic hydrolysis (Huijgen, Reith, and den Uil 2010).

2.3.4 Biological pretreatment methods

Biological pretreatment utilizes the biodegradation potential of lignin-degrading fungi and is known to enhance the enzymatic hydrolysis of sugar polymers. White-rot fungi attack mainly the lignin, while brown and soft rot fungi attack the hemicellulose and cellulose (Galbe and Wallberg 2019; Tian, Zhao, and Chen 2018; Anukam and Berghel 2021; Tan et al. 2021). During the biodegradation of lignocellulose, a complex synergetic effect of a variety of hydrolytic or oxidative enzymes such as cellulases, hemicellulases, pectinases or ligninases takes place in the cell walls, which are used by the bacteria as well as fungi for energy and nutrient production from the cell wall polymers (Tan et al. 2021). For the energetic and material use of biomass, suitable strains are also industrially produced, which are able to produce high contents of extracellular enzymes for the decomposition of biomass (e.g., Aspergillus and Trichoderma) (Schimpf 2014; Tan et al. 2021; Sindhu, Binod, and Pandey 2016).

The review paper by Tan et al. (2021) reported that numerous white-rot fungi were studied on different straw biomasses and showed excellent delignification rates. For example, in Talebnia et al., 35% reducing sugars were obtained after a five-week pretreatment with *Pleurotus ostreatus* compared to untreated wheat straw (12% reducing sugars). In Cianchetta et al. (2014), wheat straw was pretreated with the strain *Ceriporiopsis subvermispora* for 10 weeks, and digestibility and fermentable sugars were increased up to 60% compared to untreated straw. The fungal pretreatment with *Trametes versicolor* applied in Akyol et al. (2019) had a positive effect on biomethanization of lignocellulosic biomass crops such as wheat and resulted in a remarkable cellulose degradation of 80%.

The parameters pH, temperature, substrate composition, water activity, phenolic component content and enzyme concentration are crucial in the enzymatic bioconversion of lignocellulose (Schimpf 2014). Biological pretreatments have high saccharification efficiency and environmental friendliness, but at the cost of a long pretreatment cycle (Schimpf 2014; Anukam and Berghel 2021; Galbe and Wallberg 2019; Tian, Zhao, and Chen 2018; P. Kumar et al. 2009). However, the treatment time can be shortened by combining, for example, biological with chemical and/or physical treatment processes (Tan et al. 2021; Sindhu, Binod, and Pandey 2016; Theuretzbacher et al. 2015; Zhong et al. 2011).

A biological treatment in combination with LHW, moderate physical or chemical treatment has also been reported in Sindhu et al. (2016). The main advantage is that fungal treatment in combination with other technologies can shorten the operation time and increase the yield of enzymatic hydrolysis compared to treatment alone. In the study of Wang et al. (2012), it was observed that the combination of *Populus tomentosa* with LHW removed 92.33% hemicellulose and achieved the highest glucose yield, which corresponded to a 2.66-fold increase in glucose yield compared to LHW.

In conclusion, the mechanisms of biological pretreatment are still unknown due to the complex structure of microorganisms, and therefore it is necessary to further investigate the mechanisms of pretreatment with different sources of microorganisms (A. K. Kumar and Sharma 2017; Tan et al. 2021; Galbe and Wallberg 2019; Anukam and Berghel 2021; Tian, Zhao, and Chen 2018; Palvasha et al. 2021; Sindhu, Binod, and Pandey 2016). In addition, it is necessary to establish a suitable combination of physical, chemical and biological pretreatment, which could be more efficient than a single treatment method, because the combined pretreatment can have synergistic functions in straw biomass conversion and enzymatic hydrolysis as well as compensating some disadvantages of a single treatment.

2.3.5 Evaluation of pretreatment methods related to the scope of the thesis

In this chapter, the presented pretreatment processes, which can be assigned to the effect categories physical, physical-chemical, chemical and biological, are each evaluated in terms of their different advantages and disadvantages in relation to the research scope of this thesis. Currently, there is no single pretreatment technology that can fully realize the economic, environmentally friendly and efficient treatment of biomass.

An advantage of aforementioned physical pretreatment methods is generally the absence of toxic substances that could form during the process (Sindhu, Binod, and Pandey 2016; Galbe and Wallberg 2019; A. K. Kumar and Sharma 2017; Zhong et al. 2011; Anukam and Berghel 2021; Tan et al. 2021; Tian, Zhao, and Chen 2018; P. Kumar et al. 2009). Both conversion routes, biochemical and thermo-chemical, require prior (mechanical) comminution to remove restrictions on mass and heat transfer (Anukam and Berghel 2021). Therefore, initially a high energy input for mechanical comminution must be accepted in order to achieve the highest possible degree of utilization of the lignocellulose.

Pretreatment by extrusion has many advantages, such as shorter reaction time, lower cost, higher solids loading, easier control and moderate conditions, but is associated with high energy consumption. Moreover, twin-screw extrusion preferably requires crushed material, which further increases energy consumption due to upstream processes. Nevertheless, extrusion is an environmentally friendly process as no additives or chemicals are required (Duque, Manzanares, and Ballesteros 2017).

While microwave treatment can significantly reduce reaction time, it is associated with high cost and many equipment requirements (especially specific reactors). It also requires compatible downstream processes that usually need additional comminution, which further increases energy demands (Tan et al. 2021). Microwave-assisted alkali pretreatment appears to be an effective combination for wheat straw pretreatment, but is associated with difficulties in recycling the alkalis because a significant amount remains in the biomass mixture (Subhedar and Gogate 2016; Dinh Vu et al. 2017; Tan et al. 2021). Apart from the high energy requirement, the environmental compatibility is questionable as a result.

Ultrasound is a relatively new technique for pretreatment of lignocellulosic biomass, and some adverse effects have been reported (A. K. Kumar and Sharma 2017). Research is currently being conducted on suitable combinations with other straw biomass pretreatment processes (Palvasha et al. 2021).

Thermo-chemical conversion of biomass requires grinding for densification, pelletization, and in most cases torrefaction prior to thermo-chemical conversion. This pretreatment method uses heat to initiate changes that lead primarily to improved combustion properties of the biomass. However, a major disadvantage of torrefaction is its inability to remove the lignin content of lignocellulosic materials, leaving the cellulose content of the material inaccessible. Other weaknesses include high energy consumption and the prohibitive cost of commercial scale-up (Anukam and Berghel 2021).

During physical-chemical pretreatment, a high degree of degradation of hemicellulose as well as good hydrolyzability of the cellulose is possible at comparatively low operating costs. Furthermore, the temperature effect in particular leads to the formation of degradation products, which can inhibit a biogas process (Tian, Zhao, and Chen 2018; P. Kumar et al. 2009; Galbe and Wallberg 2019; A. K. Kumar and Sharma 2017). Moreover, thermal treatment is also associated with high energy consumption. Besides, disadvantages arise from the use of chemicals, since chemicals can lead to corrosion problems in the equipment and must be disposed of at great expense (P. Kumar et al. 2009; Galbe and Wallberg 2019; Tan et al. 2021). Compared to other pretreatment methods such as AFEX or $scCO_2$, LHW pretreatment is characterized by a comparatively simple process setup (Tan et al. 2021; Galbe and Wallberg 2019; J. A. Pérez et al. 2008; Li et al. 2020; Lau et al. 2010). In addition, most of the hemicellulose, parts of the cellulose and parts of the lignin are degraded and dissolved, minimizing the formation of degradation products that inhibit the growth of fermentative microorganisms (Tan et al. 2021; J. A. Pérez et al. 2008; José A. Pérez et al. 2007). Due to these properties, the LHW process is, despite the comparatively high energy demand, an efficient and environmentally friendly technology in the field of physical-chemical pretreatment methods. Furthermore, good results have been achieved in recent years when the LHW process has been combined and enhanced with biological processes, thus increasing environmental compatibility by eliminating the use of chemicals (W. Wang et al. 2012; Sindhu, Binod, and Pandey 2016).

The advantages of chemical pretreatment are high degradation of hemicellulose, high monosaccharide production, high hydrolyzability of cellulose, low operating costs, and low energy consumption (Tian, Zhao, and Chen 2018; Tan et al. 2021; Zhao et al. 2017; Raita et al. 2017; Galbe and Wallberg 2019; A. K. Kumar and Sharma 2017). A disadvantage, however, is the formation of degradation products (Raita et al. 2017; Borand and Karaosmanoğlu 2018; Zhao et al. 2017; Huijgen, Reith, and den Uil 2010). As inhibitors in the subsequent fermentation processes, these can interfere with the digestion of the biomass. In addition, the use of chemicals can lead to corrosion problems in equipment components. The effort required to recycle the chemicals is associated with high energy input and costs (Tan et al. 2021; Galbe and Wallberg 2019; Tian, Zhao, and Chen 2018). The necessary disposal of the non-recyclable chemicals is another disadvantage of this pretreatment method. The environmental impact must be questioned, a balance between the process outcome and the environmental impact has to be found. Organo-Solv treatment has the greatest advantage of the chemical processes, as high cellulose separation and hemicellulose fractionation efficiency, high lignin dissolution and lower by-product production can be achieved. Nevertheless, the disadvantage of solvent recycling and further fractionation remains (Tan et al. 2021).

The previously mentioned methods of pretreatment require costly equipment, a high consumption of energy, and the use of harmful chemicals that lead to environmental pollution. Biological pretreatment is characterized by low operating costs and low energy consumption (Tan et al. 2021). Due to the high specialization of various microorganisms on the decomposition of the individual biomass components, a good degradation of hemicellulose, a high monosaccharide production as well as a high hydrolizability of the cellulose are possible. Compared to the other pretreatment methods, however, biological pretreatment requires significantly more time (usually weeks to months). In addition, process control is complex, since successful decomposition depends on the substrate used and the ability of the respective microorganisms to degrade it. To improve the time required and the degradation capability, a combination with other pretreatment methods is necessary. Table 2 provides an overview of the methods described and their advantages and disadvantages.

Table 2: Overview of pretreatment methods; comparison of the advantages and disadvantages.

Category	Approach for method	Advantages	Disadvantages
Physical-mechanical	Hammer mills, defibers, extruders, impact reactors, perforated disc shredders, dissolvers, choppers	No formation of degradation products, simple process effort, increase in surface area for enzymatic saccharification	High energy consumption, incomplete decomposition of lignocellulose
Physical	Ultrasonic device, microwave appliance, pyrolysis furnace	Increased delignification and enzymatic saccharification	High equipment requirements, high energy requirements
Physical-chemical	Fluidized bed reactors, autoclaves, supercritical fluid reactors	High degradation of fibers, high hydrolyzability, moderate operating costs	Recycling of chemicals, high equipment requirements, degradation products, corrosion problems, high energy consumption
Chemical	Concentrated or diluted acids, alkalis, oxidizing agents or organic solvents	High degradation of fibers, high hydrolyzability, moderate operating costs, moderate energy consumption	Recycling of chemicals, high equipment requirements, degradation products, corrosion problems
Biological	Microorganisms, bacteria, fungi, enzymes	Moderate degradation of fibers, high hydrolyzability, low energy consumption, eco-friendly	Vulnerable process control, long reaction time

The comparison of the different methods has shown that from a technical point of view the Organo-Solv method is outstanding. In addition, the LHW method seems to have great potential and, as mentioned, is one of the oldest methods for pretreatment. Accordingly, these two methods were selected as reference methods for an LCA comparison in order to be able to classify the developed method in this thesis.

2.4 Implications for the evolution of combined pretreatment methods

Although combined pretreatment has achieved some satisfactory results, combined treatment technology still needs further development to reach its full potential and achieve efficient as well as eco-friendly biomass pretreatment. In view of the objective and the research question of this thesis, a chemical-free pretreatment process with a biological approach for lignocellulosic biomass should be developed and its environmental friendliness investigated by means of LCA. Therefore, the use of specially cultivated microorganisms should be avoided in order to develop a pretreatment concept that is as environmentally friendly and cost-effective as possible. However, prior mechanical comminution is a basic prerequisite for further pretreatment, as described above. To achieve the objective of this thesis, biogenic residues were integrated into the pretreatment concept as natural additives which, due to their properties, have lignocellulose-degrading capabilities and combine the advantages of the different pretreatment categories. Compost was selected as a natural source of microorganisms and liquid digestate was chosen as an equivalent ammonia solution based on $NH4^+$-N concentration.

Of particular interest was compost and its ability to degrade lignocellulosic materials (during material conversion in the composting process). As can be seen in Figure 12, the mesophilic start-up phase produces mesophilic organisms such as fungi and bacteria, which reach their maximum growth temperature between 20 and 42 °C (Kosowski 2013; Trautmann and Krasny 2014). In the first days, there is intensive bacterial activity with a high metabolic rate and the breakdown and mineralization of easily degradable organic substances (e.g., sugars, amino acids, fats). During the subsequent thermophilic rotting phase (45 – 70 °C), the temperature stagnates at about 50 °C. The organism populations change to thermotolerant, moderately thermophilic as well as thermophilic organisms (mainly bacteria), whose optimal growth temperature is above 40 °C and which can grow up to a limiting temperature of approx. 70 °C.

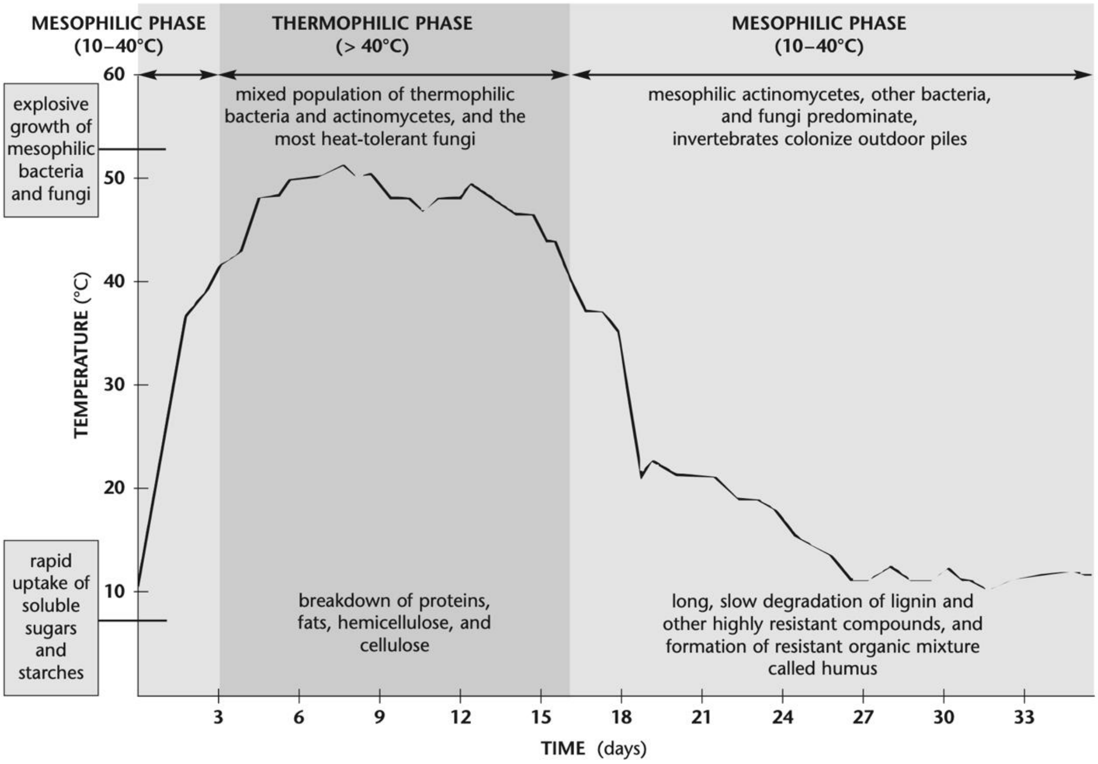

Figure 12: Simplified scheme for the course of the material conversion rates in the composting process (Trautmann and Krasny 2014).

Further degradation of complex organic substances (e.g. lignin, cellulose, proteins, and hemicellulose) takes place. During the mesophilic cooldown phase, temperatures fall below 45 °C. This leads to a renewed increase in mesophilic organisms. Further cleavages of poly- and disaccharides to monosaccharides occur. As the composting temperature continues to drop, further degradation of hard-to-degrade compounds such as lignin and the buildup of humic substances begins (Trautmann and Krasny 2014; Kosowski 2013).

In this thesis, the process parameters were adapted to the milieu conditions in the composting process. The adaptation intended to achieve optimal environmental conditions for the biological decomposition of the wheat straw by the microorganisms in the compost mass. Biological decomposition was promoted by further combining it with physical (hammer mill, defibers, extruder), chemical (liquid digestate), and physical-chemical (autoclave) pretreatment. Mechanically shredded wheat straw was mixed with water or liquid digestate to adjust a dry matter (DM) content between 15% and 30%. To ensure the effect of the selected additives, the raw material was first sterilized by autoclaving. Thermal (autoclave) treatment of the samples was performed at 120 or 140 °C (20 min). Subsequently, autoclaved substrate was mixed with compost from the thermophilic phase and incubated under aerobic conditions at 25 °C and anaerobic conditions at 55 °C for 14 days each. Thus, different milieu conditions (aerobic, anaerobic and mild-mesophilic as well as thermophilic) were created which could be compared in subsequent analyses.

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3 Comparative Life-Cycle-Assessment of pretreatment processes for the production of biofuels from lignocellulosic residues

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3.1 Abstract

Residual and waste materials from agriculture and forestry often contain high amounts of lignocellulose, which counteracts its use to produce both bioenergy and bio-based products. These raw materials usually have to be pre-treated. Then further processing to produce chemicals for bio-based products, bioenergy or second-generation biofuels supply can take place. In this study within the framework of a Life-Cycle-Assessment processes for the pretreatment of lignocellulosic materials, primarily wheat straw, are examined for their respective environmental influences. The aim of the research is to determine potential environmental impacts and to identify possible weak points in the procedures. In addition to the technical and environmental evaluation of the processes, the efficiency regarding production of biogenic substitutes, i.e. the amount of necessary derivatives for the production of fuels, was also evaluated during this trial. For this purpose, the acetone-based pretreatment process and the water-based Liquid-Hot-Water pretreatment were analyzed within a Life-Cycle-Assessment. To calculate the most relevant factors the method *ReCiPe 2016 v1.1 midpoint (H)* and the impact factors global warming potential, eutrophic potential, human toxicity, and fresh-water toxicity were chosen. Results have shown that the Liquid-Hot-Water process, which uses water as a solvent with temperatures over 200 degrees (Celsius) and a pH value of 5.6, is more environmentally friendly than the acetone-based process. Additionally, the correlation to post treatment processes like the filtration was visualized. So, vacuum filtration systems seem to be less polluting than paper-based filtration systems. Advantage of the acetone-based treatment is that the fractions have a better separation, so a better quality and especially accessibility of the lignin fractions is realized. Liquid-Hot-Water pretreatment achieves a better mass balance, especially in terms of a following biofuel production, except for its mixed cellulose and lignin fraction, which is a disadvantage.

Keywords: lignocellulose material, wheat straw, life-cycle-assessment, pretreatment, bio-fuels

3.2 Introduction

Sustainably supplying food, energy, and raw materials without harming the environment is a central task of society. As a source of energy and raw material for many chemical industrial products, crude oil is currently the dominant raw material in the global economy. In Germany alone, approximately 100 million tons of fossil crude oil are used annually. 85% are used in the transport and energy sectors and the rest in the chemical industry (VCI 2017).

Both climate change and the depletion of fossil raw materials are increasing the need for research in developing processes for the use of biomass as an alternative raw material basis in the energy and chemical industries. A sustainable and efficient use of fiber-rich or straw-like residues as material and energy-supplying substrates can lead to the simultaneous substitution of fossil raw materials and CO_2 savings (LANUV 2014). Due to the high content of lignocellulose, residual and waste materials from agriculture and forestry must first be treated in a pre-treatment process for further processing (Haase 2012; Kaltschmitt and Hartmann 2016; BMELV 2014).

In the life cycle analysis carried out here, processes for the holistic pre-processing of lignocellulose-containing materials in the sense of a biorefinery were examined with regard to their environmental influences. The use and efficiency of fiber-rich residual materials, such as wheat straw, is intended to be increased through innovative process combinations (BMELV 2014). Wheat straw is partly used as bedding and as humus and only a very small proportion is used for raw material utilization (LANUV 2014). Germany produces about 35 million tons of straw per year. In the future, more biomass per hectare can be produced due to increasing agricultural productivity (BMELV 2014). Depending on the humus balance method used, Germany's sustainable straw potential that can be used as a raw material amounts to 8 to 13 million (t/a fresh matter). This corresponds to 27 to 43% of the theoretical straw potential or 114 to 186 (PJ/a) (Kaltschmitt and Hartmann 2016).

A part of the targeted technical development of sustainable process chains for biomass use is an accompanying ecological evaluation of the processes developed on a laboratory scale. Based on scenario analyses, it is possible to define a process design that is ecologically most efficient. The focus of this work is therefore on laboratory-scale processes already known in the literature that deal with processing wheat straw. Carrying out a Life-Cycle-Assessment (LCA), which was designed according to the requirements of DIN EN ISO 14040, serves as the basis for the analysis (ISO 2006). The technical implementation was carried out with the help of the LCA *GaBi* program.

The aim of this work is to make statements on potential environmental impacts. In addition, possible weaknesses in the process design were identified by comparing the pretreatment processes. With the help of a comparison matrix according to VDI 2225, which was merged separately with the results of the Life-Cycle-Assessment, further factors for the process evaluation were taken into account (VDI 1997). According to VDI 2225, the definition and weighting of evaluation criteria took place before the actual comparison (VDI 1997). Because fractionation processes were investigated to provide resources for the production of biofuels, the production of fermentable sugars was considered more relevant than the provision of lignin. It was also possible to assess the efficiency of the processes in relation to the quantitative provision of biogenic substitute products for biofuel production.

3.3 Material and methods

3.3.1 Selection of the procedures to be compared

The fractionation of wheat straw requires high delignification, hemicellulose solubilization, and glucose yield under environmentally friendly process conditions (Haase 2012; Ludwig 2014). The aim of the comparative LCA is to investigate holistic processes that treat all products as possible target products. Therefore, only the chemical and physico-chemical processes can be considered due to the qualitative, quantitative, and ecological results (Haase 2012; Ludwig 2014; Uihlein and Schebek 2009). When selecting the processes, it is important to consider diversity in the process steps in order to make statements about the advantages of water- or chemical-based processes. The Liquid-Hot-Water process (LHW), which is representative of physico-chemical water-based processes, and the acetone-based Organo-Solv process, which is representative of processes based on alcoholic solvents, were therefore selected for the comparison (Ludwig 2014; Kumar and Sharma 2017).

(1) The Liquid-Hot-Water process

In Pérez et al. (2007) the focus was on the fractions cellulose and hemicellulose and various process parameters were simulated in order to achieve the highest possible cellulose yield or hemicellulose hydrolysis. The composition of the used wheat straw with a moisture content of 6.8% is shown in Table 3.

Table 3: Composition of wheat straw in the LHW process, from data from Reynolds et al. (2016) and Pérez et al. (2007).

Component	Concentration (in % DM)
Extractives	14.7 +/- 0.1
Cellulose	37.4 +/- 0.0
Hemicellulose	27.7 +/- 1.7
Acid-insoluble lignin	15.6 +/- 0.4
Acid-soluble lignin	1.8 +/- 0.6
Ash	4.8 +/- 0.2

The main focus in Reynolds et al. (2016) was on lignin and the associated parameters. The combination of both studies enables a detailed and sufficient data situation for a life-cycle analysis due to the compatibility of the results. Before the LHW process, the wheat straw was milled into particles of < 2 cm using a hammer mill. The water-straw mixture was fed into a Hastelloy-C reactor with a volume of 0.5 liters in a ratio of 1:20 or 1:10. With a speed of 2 to 4 (°C min^{-1}) and constant mixing (600 min^{-1}), the mixture was heated to the process temperature (200 °C), changing the environment of the water to the acid range with a pH value of about 5.6. After reaching the process temperature and 30 bar pressure, the water-straw mixture was kept at the temperature level for 40 minutes. The reactor was cooled to 70 °C within 6 minutes by water cooling. Afterwards, the reactor was cooled down to 50 °C in a relaxed state. Then the liquid and solid fractions were separated using a vacuum filter. The mixed solid cellulose and lignin fraction was washed with water (Reynolds et al. 2016; Pérez et al. 2007).

(2) The Organo-Solv process based on acetone

In Huijgen, Reith, and den Uil (2010), pretreatment processes based on acetone as an organic solvent were analyzed under different process parameters such as temperature, acetone concentration, and retention time. The composition of the used wheat straw with a moisture content of ~ 8% is shown in Table 4.

Table 4: Composition of wheat straw in the Aceto-Solv process, from data from Huijgen, Reith, and den Uil (2010).

Component	**Concentration (in % DM)**
Extractives (water, ethanol)	13.2 +/- 1.2
Cellulose	34.6 +/- 1.0
Hemicellulose	24.3 +/- 0.9
Acid-insoluble lignin	15.1 +/- 0.1
Acid-soluble lignin	1.0 +/- 0.0
Ash	8.5 +/- 0.2

Acetone-based pretreatment processes represent an efficient process for fractionation of wheat straw, because of improved delignification compared to ethanol and at the same time an increased lignin quality and metabolism of sugar derivatives during enzymatic hydrolysis (BMELV 2014; Uihlein and Schebek 2009; Borand and Karaosmanoğlu 2018). This kind of Organo-Solv process is also called Aceto-Solv. According to Huijgen, Reith, and den Uil (2010) the process conditions with an acetone-water solution in a ratio of 1:1 are optimal with a residence time of one hour in the reactor at 205 °C. The material was also processed with a hammer mill in < 2 cm particles.The straw was mixed with an acetone-water solution (ratio 1:1), in a mixing ratio of 14.2 g solution per gram dry matter straw, in a 0.5 liter C-Hastelloy with anchor agitator (100 min^{-1}). The reactor was heated for 49 minutes (3.55 °C min^{-1}) to an operating temperature of 205 °C, which was maintained isothermally for 60 minutes. The reactor was cooled down to 40 °C (4.69 °C min^{-1}) for 35 minutes. Then the mixture was separated into a liquid and a solid fraction in a Whatman-Type-3 paper filter. The solid fraction, mainly containing cellulose and hemicellulose, was washed with an acetone-water solution in the same mixing ratio (acetone:water 1:1 or solution:dry mass wheat straw 14.2:1) and then dried overnight in a vacuum oven at 50 °C to prepare for the analysis of the ingredients. The remaining washing solution was added to the liquid fraction for further lignin recovery (Huijgen, Reith, and den Uil 2010).

3.3.2 Definition of the framework for primary refining processes for the fractionation of lignocellulosic raw materials

The scope of the assessment is based on the pretreatment processes described above. The following assumptions were therefore derived for a consistent and resilient framework:

- The production of the raw material wheat straw and the resulting emissions and influencing variables in the impact categories were taken into account as input for the elementary flow of wheat straw.
- The transport of wheat straw has been taken into account. Any elementary flows during the process, such as lost straw, were not taken into account. However, elementary flows in the form of emissions during transport were taken into account by impact categories and do not find a separate item in the framework of the assessment.
- All incoming and outgoing energy and mass flows during the process were recorded.
- All platform products were considered.
- The efficiencies of the machines used, such as reactors, mills, separating filters or coolers, were not taken into account in order to avoid endangering the comparability of the processes. Where necessary, the physically required energy flows were calculated. For example, the required thermal energy of the reactors was calculated with the corresponding heat capacity of the respective materials. If a separate consideration of the necessary energy was not possible, e.g., during the comminution of the straw, the energy flows were determined by own experiments.

- The analysis of the processes ends with the production of lignin, cellulose (glucose) and hemicellulose, C5 and C6 saccharides, and their degradation products.

Based on these assumptions and in accordance with ISO 14040 and VDI 6310, the following general framework for the selected processes was defined in Figure 13.

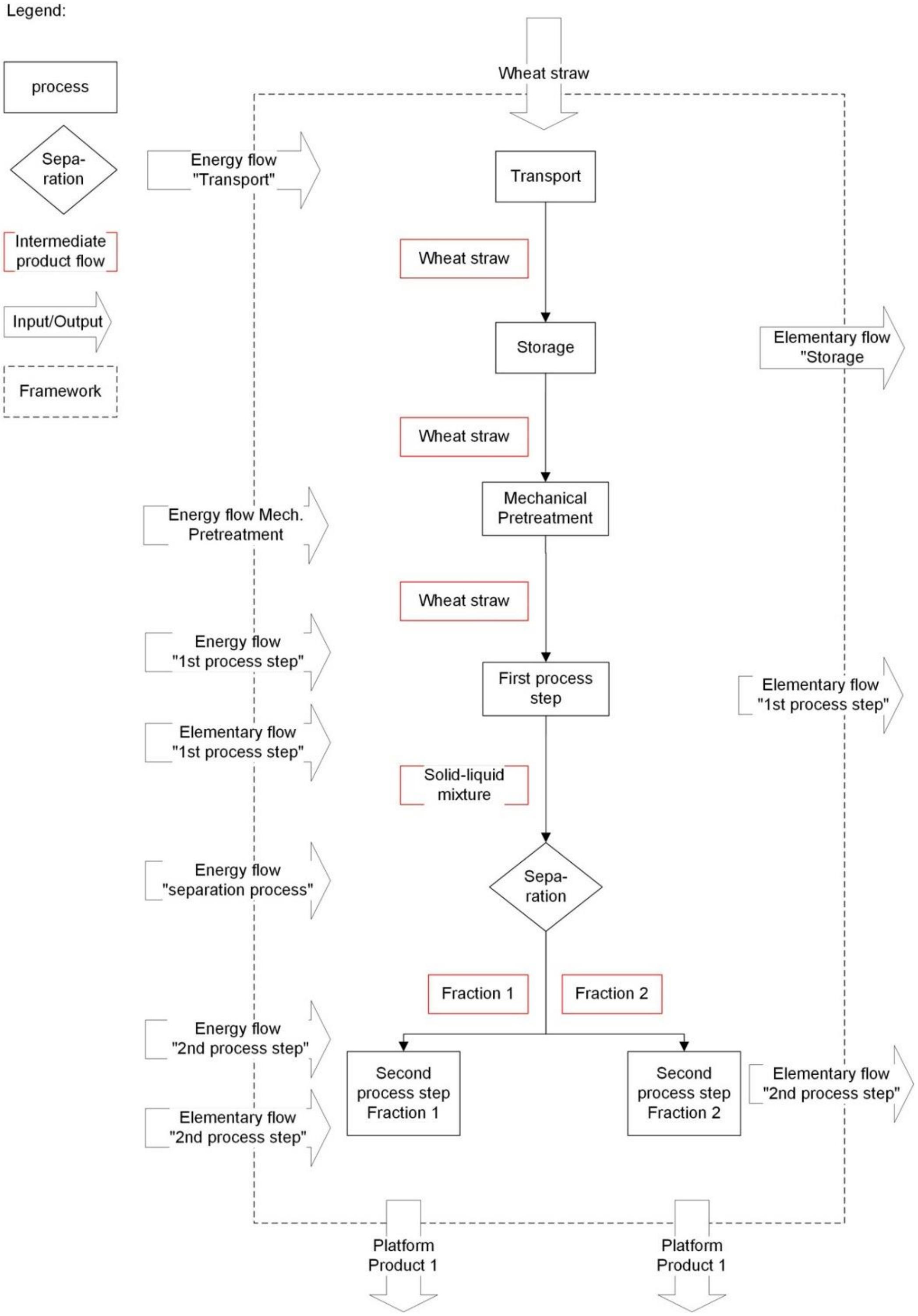

Figure 13: Framework of the Fractionation Processes according to ISO 14040 and VDI 6310.

3.3.3 Quantifying the processes

Because the fractionated lignocellulose components differ both in quantity and quality, the reference per kilogram of wheat straw dry matter (DM) or dimension (1 kg DM wheat straw) was applied as the functional unit for the Life Cycle Inventory, according to Haase (2012) and Uihlein and Schebek (2009). In order to ensure equal conditions for the pretreatment processes investigated, the elementary and energy flows were equally included both in the provision of the raw material wheat straw and in its transport. For the quantification of the processes, the following important process variables in Table 5 were identified according to Huijgen, Reith, and den Uil (2010), Reynolds et al. (2016) and Pérez et al. (2007) and the framework of the assessment in Figure 13.

Table 5: Identified relevant process variables for the quantification of LHW and Aceto-Solv

Process stage	LHW	Aceto-Solv
1	Consumption of resources and energy during the production of wheat straw	Consumption of resources and energy during the production of wheat straw
2	Use of fuels during the transport of wheat straw	Use of fuels during the transport of wheat straw
3	Storage of wheat straw	Storage of wheat straw
4	Crushing of wheat straw	Crushing of wheat straw
5	Water injection into the reactor	Introduction of acetone/water into the reactor
6	Contribution of energy for heating, temperature maintenance and pressure provision in the reactor	Energy input for heating in the reactor
7	Water is brought in for cooling	Waste heat removal during cooling
8	Energy input during vacuum filtration	Introduction of a paper filter during separation filtration
9	Water is added to wash the solid fraction	Acetone/water is added to wash the solid fraction
10	Removal of the washing solution	Introduction of a paper filter during separation-filtration of the solid fraction after washing
11	Removal of reaction water	Water is introduced to wash the liquid fraction
12	Analysis of the composition and quantity of fractions	Energy input for the centrifugation of the liquid fraction
13	-	Removal of the washing solution and the acetone solvent
14	-	Analysis of the composition and quantity of fractions

The calculations related to the functional unit (1 kg DM wheat straw) were carried out on the basis of sources and, if necessary, completed by tests carried out in accordance with Beuel et al. (2018).

3.3.4 Selection of impact categories

The evaluation method *ReCiPe* according to the Midpoint method H was chosen because of its flexibility and the combination of a long-term and realistic approach (Haase 2012; Ludwig 2014; Uihlein and Schebek 2009). In addition, *ReCiPe* has a good data availability in the field of biological-chemical processing due to the EcoInvent database (Haase 2012; Goedkoop and Huijbregts 2013; Lask et al. 2019; Shadbahr, Zhang, and Khan 2015). The potentials are given in different equivalents, listed as units in Table 6, which make the impact potential measurable and comparable.

Table 6: Midpoint impact categories of the *ReCiPe* method according to Goedkoop and Huijbregts (2013).

Characterization factor	**Unit**	**Abbreviation**
global warming potential	kg (CO_2 to air)	GWP
freshwater eutrophication potential	kg (P to freshwater)	FEP
human toxicity potential	kg (1.4-DCB to urban air)	HTP
freshwater ecotoxicity potential	kg (1.4-DCB to freshwater)	FETP

3.4 Results and discussion

In all categories, the Aceto-Solv process has significantly more negative environmental impacts than the LHW process. The results obtained with *GaBi* are listed in Table 7.

Table 7: Environmental impacts of pretreatment processes according to *ReCiPe* 2016 v1.1 Midpoint (H)

Impact category	**Unit**	**LHW**	**Aceto-Solv**
GWP	kg CO_2-equiv. kg^{-1} DM^{-1}	12.8	67.4
FEP	kg P-equiv. kg^{-1} DM^{-1}	4.91 x 10-5	0.00136
HTP	kg 1.4-DCB-equiv. kg^{-1} DM^{-1}	0.249	8.58
FEP	kg 1.4-DCB-equiv. kg^{-1} DM^{-1}	0.00875	0.0515

3.4.1 Global warming

The climate impact of the Aceto-Solv process is about five times higher than the one of the LHW process. The higher global warming potential in the Aceto-Solv process can be explained by the use of acetone as a solvent (Prasad et al. 2016; Borand and Karaosmanoğlu 2018; Lask et al. 2019). In addition, compared to the LHW process, there is the double energy input for heating the reactor and additionally for centrifuging the liquid fraction (Prasad et al. 2016). In the LHW process, the heating of the reactor is the only significant parameter that has an influence on emissions (Lask et al. 2019; Prasad et al. 2016). In the Aceto-Solv process, the required process energy causes 50% of the emissions. In addition, acetone is required, which causes about 40% of the emissions, as these are very energy-intensive processes (Borand and Karaosmanoğlu 2018; Prasad et al. 2016; Raita et al. 2017).

3.4.2 Fresh water eutrophication

The same applies to the fresh water eutrophication potential, which is about two and a half times higher in the Aceto-Solv process than in the LHW process. The LHW process mainly produces emissions during wheat production (about 80% of all emissions) (Uihlein and Schebek 2009; Prasad et al. 2016). In the Aceto-Solv process, these only have a marginal share compared to those of paper production, required for producing the filter. Here, the use of a paper-based filter is mainly responsible for the emissions. The use of a vacuum filter system as in the LHW process has a lower overall energy demand in comparison.

3.4.3 Human toxicity

Within the scope of the human toxicity potential impact category, the emissions arising from the provision of the process energy are decisive for the level of the hazard potential for the LHW process. A small share of the emissions (< 5%) occurs during the provision of wheat straw. The use of a paper filter is essential for the increased risk potential of the Aceto-Solv process. Only small parts of the total emissions are due to the use of acetone, electricity, and wheat production (in total < 5%) (Borand and Karaosmanoğlu 2018). The total hazard potential of the Aceto-Solv process is almost 35 times higher.

3.4.4 Fresh water toxicity

In contrast to the human toxicity potential, the fresh water toxicity potential of the LHW process results from the removal of acetyl or acetic acid, which only make up a small part of the fractions, but about 30% of the total emissions (Prasad et al. 2016). Almost 70% relates to electricity supply and only a small proportion to wheat straw production. In the Aceto-Solv process, the paper filter is repeatedly the main emitter with around 60%. The remaining 40% is accounted for by the provision of process energy (~ 20%), the removal of waste products, and the provision of acetone and wheat straw (~ 10% each) (Borand and Karaosmanoğlu 2018; Prasad et al. 2016). The emissions of the Aceto-Solv process are also significantly higher here with a sixfold increase in emissions.

3.4.5 Extended comparison with utility value analysis

When the comparative matrix according to VDI 2225 is considered (Table 8), the Aceto-Solv process with a rating of 47% scores worse than the LHW process with a rating of 97%.

Table 8: Comparative analysis of fractionation processes according to VDI 2225, technical criteria in light grey and ecological criteria in dark grey.

		Weighting		**Raw data**		**Unit**
		LHW	**Aceto-Solv**	**LHW**	**Aceto-Solv**	
Quantity Glucose	**20%**	100%	78%	417	325	g kg^{-1} DM^{-1}
Quantity Xylose	**15%**	98%	100%	59	60	g kg^{-1} DM^{-1}
Quantity Lignin	**5%**	100%	81%	199	161	g kg^{-1} DM^{-1}
Quality/Availability Lignin	**5%**	40%	100%	2	5	
GWP	**20%**	100%	19%	12.8	67.4	kg CO2-equiv. kg^{-1} DM^{-1}
EP	**10%**	100%	4%	0.0000491	0.00136	kg P-equiv. kg^{-1} DM^{-1}
HTP	**10%**	100%	3%	0.249	8.58	kg 1.4-DCB-equiv. kg^{-1} DM^{-1}
FETP	**15%**	100%	17%	0.00875	0.0515	kg 1.4-DCB-equiv. kg^{-1} DM^{-1}
	Result	**97%**	**47%**			

Reasons are the results of the Life-Cycle-Assessment and the fact that the Aceto-Solv process provides comparatively less sugar and sugar derivatives than the LHW process (Prasad et al. 2016). So, the LHW process is recommended when the goal is the provision of fermentable sugars for biofuel production. Only if the separate lignin supply in good quality would be rated higher, could the weaknesses of the Aceto-Solv process be compensated (Borand and Karaosmanoğlu 2018; Lask et al. 2019; Prasad et al. 2016; Raita et al. 2017). As Shadbahr, Zhang, and Khan (2015) have noted, modifications in the process design could lead to a lower environmental impact. In this case, using a different filtration method would also improve the Aceto-Solv process from an environmental point of view in nearly all impact categories.

3.5 Conclusion

The life cycle analysis method is suitable for identifying ecological “weak points” in process design. In addition, it was expected that acetone as a solvent would have a significantly more negative impact on the ecological balance than water. However, the results have shown that the use of a paper filter system has a much stronger impact on the LCA.

For future process developments, a model was created in this paper to apply eco-design principles already during the process chain development. Moreover, the knowledge gained about process design on a laboratory scale can be directly applied for upscaling the processes.

It should be emphasized that the efficiency or quality of the substances obtained are not taken into account in a purely ecological evaluation of process chains. Therefore, further criteria must be included in the evaluation, such as technical criteria on the quality and availability of the respective fractions. This requires further investigations. The results must be subjected to an economic analysis in order to clarify whether the highest possible sugar yields for biofuel production also bring the greatest economic benefit. As a result, a process chain can be defined that is both economically advantageous and ecologically sensible.

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4 Biogenic catalysis by adding compost when using wheat straw in a biorefinery concept

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4.1 Abstract

The high content of lignocellulose limits the biodegradability of wheat straw for bioenergy production. To counteract this, a thermo-biological pretreatment was applied to improve the utilization of lignocellulose biomasses for a biorefinery concept. The use of compost assured the growth of cellulose-degrading anaerobic microorganisms under thermophilic conditions. Results revealed a lignocellulose material degradation in all samples. Moreover, the combination of a thermo-biological pretreatment under thermophilic conditions created a synergistic effect that accelerated the biomethanization of wheat straw due to the sugar compounds released during the biogenic catalysis.

Keywords: biorefinery; biomethanization; biological pretreatment; lignocellulose material; wheat straw

4.2 Introduction

The rising of worldwide energy demand and availability of fossil fuels increases the environmental impact caused by emission of greenhouse gases (Sawatdeenarunat et al. 2015). Competition between the food and bioenergy markets and rising population growth have led to a debate about "food or fuel". In this context, the challenge is to explore new concepts for biomass use to counteract the choice between food and fuel while protecting the environment. To overcome this situation, biogenic residues and waste materials, which are by-products from agricultural or industrial activities, are gaining great interest as potential raw materials, mainly for the production of second-generation biofuels. The utilization of lignocellulose agricultural residues, i.e., wheat straw, is a promising feedstock for bioenergy production (Reinhold and Friedrich 2012; Schütte 2012). Straw is mainly composed of cellulose (30 – 50%), hemicellulose (20 – 35%), and lignin (15 – 20%) (Mosier et al. 2005; Reinhold and Friedrich 2012; Kaparaju et al. 2009). However, the biodegradability of such valuable biomasses is still restricted due to the high content of lignocellulose fibers, which counteracts their use in order to achieve complete digestion during fermentative processes like anaerobic digestion (AD) (Braun 2007; Stinner 2016). Therefore, suitable pretreatments need to be carried out in order to promote delignification and degradation of cellulose and hemicellulose to increase the formation of water-soluble sugars to optimize the biodegradability and production of second-generation biofuels (Stinner 2016; Schwarz 2016). Pretreatments to enhance the feedstock biodegradability can take place as physical, chemical, biological pretreatments or a combination of them (Y. Sun and Cheng 2002; Seppälä et al. 2008). Biological degradation of lignocellulosic fibers was achieved when using different microorganisms including brown-, white-, and soft-rot fungi; which improved the production of second-generation biofuels (Y. Sun and Cheng 2002; Mustafa et al. 2017; Antonczyk, Arthur, and Scherer 2016). Advantageously, biological pretreatments have low energy consumption as well as lower requirements than chemicals and fewer intermediate toxic substances form (Mutschlechner, Illmer, and Wagner 2015). Studies on mechanical pretreatments, also in combination with biological processes, have been carried out in recent years to improve the AD of straw (Mustafa et al. 2017; Antonczyk, Arthur, and Scherer 2016). In order to further improve the AD of wheat straw, recent studies have indicated that pretreatment costs can significantly be reduced by using liquid digestate (LD). LD is one of the end products of anaerobic digestion which carries abundant lignocellulose-degrading microbes, and other organic substances (e.g., amino acids, protein, and sugar) (Hagos et al. 2017). LD therefore promotes efficiency of anaerobic biogas production from lignocellulose and reduces the amount of posttreatment required. As a result, the recirculated LD for pretreatment increases the rate of bioconversion of the substrate during the AD process and additional chemical agents are not required anymore (Hu et al. 2014; Liu et al. 2019). Moreover, it was shown in different studies that the enhancement of the biodegradability of lignocellulose feedstocks using compost as a natural source of microorganisms could improve methane production (Mustafa et al. 2017; Neumann and Scherer 2011; Niu et al. 2012; Thomsen et al. 2016). Investigations were mainly based on using isolated strains from compost in order to promote degradation of the lignocellulose material (Mustafa et al. 2017; Niu et al. 2012; Thomsen et al. 2016). A recent study proposed a paradigm shift in the degradation of the lignocellulose material in wheat straw with respect to methane production, because compost contains microorganisms (e.g., bacteria and fungi) which may degrade structural carbohydrates (Bursche et al. 2018). Prior to digesting, samples were stored under anaerobic conditions at room temperature for at least 22 days. It seemed that the low improvements of AD were related to the room temperature during storage, which could not enhance the biodegradability of lignocellulosic fibers (Bursche et al. 2018).

In this context, this study investigated the effect of applying a combination of thermal, chemical, and biological pretreatments to wheat straw. Furthermore, green waste compost was used as a biological pretreatment to improve the biogenic catalysis of wheat straw to further use lignocellulose biomasses. At the same time, it was investigated how the rate of bioconversion can be increased by chemical pretreatment with LD. Instead of water (W), LD was used as an alternative additive for substrate pretreatment to consider a cascade of utilization of digestate for a biorefinery concept.

4.3 Material and methods

4.3.1 Substrate preparation, inoculum and liquid digestate

Wheat straw was used as a substrate and was obtained from a farm close to Cologne in Germany. Compost used during the biological pretreatment was obtained from the green waste composting plant in the *Leppe* waste disposal center in Lindlar (Germany). The compost was four weeks old and had a temperature of about 50 °C. The compost was sieved to remove large fractions prior to carrying out the pretreatments. The inoculum was obtained from a biogas plant using cattle manure as feedstock. Manure was stored for seven days at 37 °C as recommended by VDI 4630 prior to using it as the inoculum for AD (VDI 2014). The LD as well as the manure was collected from a biogas plant near Overath (Germany).

4.3.2 Mechanical and thermo-biological pretreatment

Wheat straw was chopped to a particle size of 2 – 4 mm. The comminution was carried out using a hammer mill with 22 kW (Type MPZ 600, *Mütek Systemtechnik)*. The chopped wheat straw (S) was mixed with water (SW) or liquid digestate (SD) to obtain a dry matter of 30. Then the samples were thermally pretreated using an autoclave at 121 °C for 20 minutes (SWa; SDa). Biological pretreatment was applied by adding green waste compost (C). Pre-treated samples were incubated aerobically at 25 °C (SCWa-I; SCDa-I) and anaerobically at 55 °C (SCWa-B; SCDa-B) for 14 days.

4.3.3 Chemical analysis

Dry matter (DM) and volatile solids content (VS) were analyzed according to ISO 18134 and ISO 18122. To investigate the effects on the fermentations process, the chemical composition of samples was analyzed using a high-performance liquid chromatograph with a *Rezex*^{TM} ROA Column (*Phenomenex LTD*, Germany), refractive index detector (RID 10A, *Shimadzu Europa GmbH*) at 60 °C with a flow rate of 0.6 ml min^{-1} and 5 mM H_2SO_4 as eluent (Sluiter et al. 2007).

4.3.4 Biomethane Potential (BMP) Determination

The methane production was assessed through laboratory-scale anaerobic batch fermentation tests to observe the effect of different pretreatments that were applied. Tests were carried out under mesophilic conditions according to standard VDI 4630 (VDI 2014). The volume of produced methane was recorded by the displacement of the liquid. Measurements were performed in triplicate for 30 days.

4.4 Results and discussion

4.4.1 Characterization of substrates

To monitor the process, DM and VS content of the substrates were assessed (Table 9). The results of lignocellulose content of wheat straw and the pretreated samples are shown respectively in Table 9 and Table 10.

Table 9: Chemical composition of used substrates ([a] no data, because not measured).

Parameters	Wheat Straw	Compost	Liquid digestate
DM (%)	92.81 ± 1.08	34.25 ± 0.90	6.96 ± 0.64
VS (%)	88.84 ± 2.45	13.98 ± 0.41	5.25 ± 0.52
VS/DM (%)	95.72 ± 1.94	40.82 ± 2.23	75.43± 1.72
Ash Content (%)	3.68 ± 1.66	6.95 ± 0.63	0.12± 0.02
Extractives (%)	5.78 ± 0.29	2.62 ± 0.12	6.50 ± 0.12
Glucan (%)	40.80 ± 0.24	7.74 ± 0.23	n.d. [a]
Xylan (%)	25.92 ± 0.56	4.15 ± 0.23	n.d. [a]
Arabinan (%)	5.51 ± 0.12	2.55 ± 0.16	n.d. [a]
Klason Lignin (%)	16.71 ± 2.64	65.34 ± 0.08	n.d. [a]

Table 10: Chemical composition of pretreated samples.

Parameters	SW	SWa	SCWa-I	SCWa-B	SD	SDa	SCDa-I	SCDa-B
Extractives (%)	3.21 ± 0.87	4.47 ± 0.96	3.75 ± 0.54	2.63 ± 0.16	4.02 ± 0.32	3.88 ± 0.49	2.58 ± 0.11	4.10 ± 0.10
Glucan (%)	38.18 ± 0.30	39.66 ± 0.75	27.12 ± 1.25	18.26 ± 1.38	34.70 ± 0.56	32.49 ± 0.37	26.26 ± 3.19	14.29 ± 2.59
Xylan (%)	25.79 ± 0.40	26.33 ± 0.64	18.49 ± 0.53	11.44 ± 1.17	23.44 ± 0.41	21.18 ± 0.29	19.36 ± 3.51	8.54 ± 1.69
Arabinan (%)	5.62 ± 0.04	5.75 ± 0.18	4.72 ± 0.09	3.73 ± 0.08	5.37 ± 0.07	5.17 ± 0.04	4.40 ± 0.33	3.32 ± 0.27
Klason Lignin (%)	18.07 ± 1.86	18.68 ± 0.76	35.64 ± 2.94	53.28 ± 3.94	19.89 ± 1.14	17.43 ± 0.64	32.15 ± 1.44	55.37 ± 4.04

As expected, wheat straw showed higher DM and VS contents (92.8% and 88.9%, respectively) than green waste compost and liquid digestate. In this assay, straw was mixed with compost during the biological pretreatment but the compost was only used as a source of microorganisms to degrade lignocellulosic biomass. LD was used to increase bioconversion and ensure additional degradation of lignocellulose. In accordance with previous results, the lignocellulose content of wheat straw was about 40% cellulose, 31% hemicellulose, and 16% lignin (Ambye-Jensen et al. 2013; Mustafa et al. 2017; Antonczyk, Arthur, and Scherer 2016). The composition of the green waste compost showed high amounts of lignin. The high lignin content of the compost material used corresponds to the rotting degree of the hot rotting phase, which suggests that the cellulose and hemicellulose content had already been degraded in the rotting process (Kosowski 2013). As a result, higher lignin content could only be observed in the SC samples compared to wheat straw during this investigation.

4.4.2 Effects of pretreatment on feedstock

Improvements on the biodegradability of the lignocellulosic materials of straw due to addition of compost as a biological pretreatment as well as the effect of addition of LD to optimize the biogenic catalysis can be observed in Figure 14.

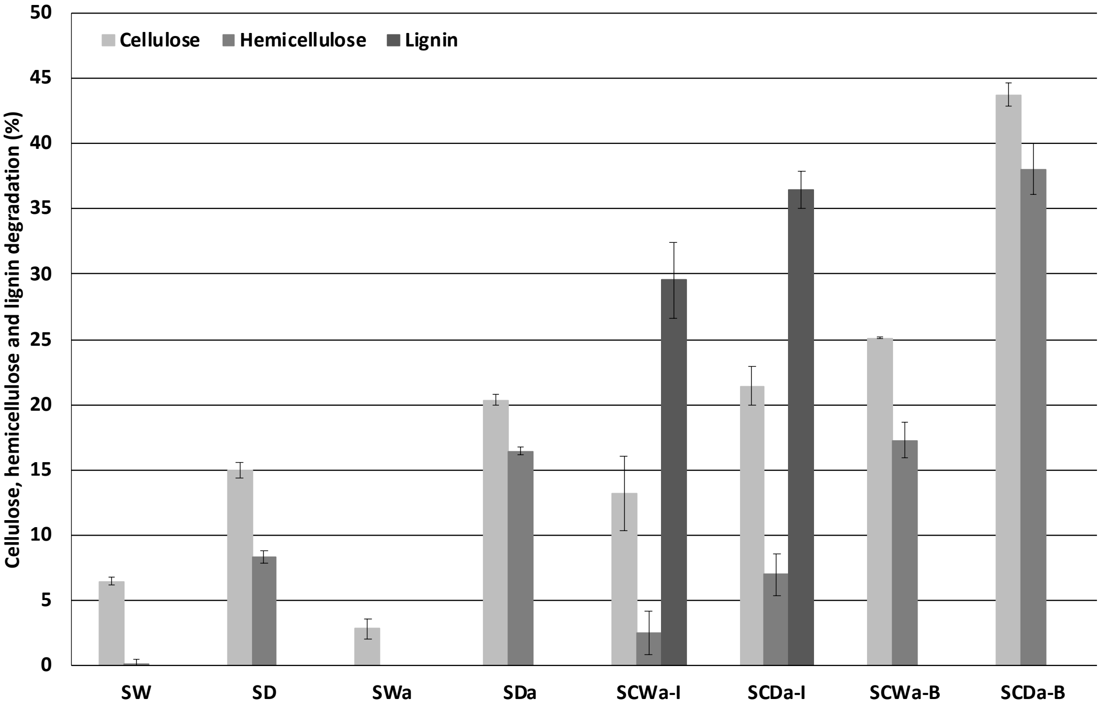

Figure 14: Cellulose, hemicellulose and Klason lignin degradation during pretreatment.
SW – straw with water; SD – straw with liquid digestate; C – sample with compost. a – autoclaved;
I – aerobically incubated for 14 days at 25 °C; B – anaerobically incubated for 14 days at 55 °C.

Different authors have already investigated the use of cellulose-degrading microorganisms for biological pretreatment to produce biogas and ethanol from lignocellulosic feedstocks (Demain, Newcomb, and Wu 2005; Crespo et al. 2012; Tuomela et al. 2000;

Zhong et al. 2011; Yuan 2011; Chang and Yao 2011). Differences in the biodegradability of lignocellulose content in the samples were observed if the samples were pretreated either with water or LD. By the addition of LD to the feedstock, a degradation of at least 15% of the cellulose and 8% of the hemicellulose was achieved (SD). In accordance with this, cellulose and hemicellulose degradation was observed when wheat straw was pretreated using LD for 7 days (Liu et al. 2019). Nonetheless, removal of lignin could not be observed in SD during this trial. Degradation of lignin only occurred, when SC samples were kept under aerobic conditions (SCWa-I and SCDa-I). Several studies have investigated the role of different fungi as a biological pretreatment to enhance biofuel production (Y. Sun and Cheng 2002; Zhong et al. 2011; Taherzadeh and Karimi 2008; Talebnia, Karakashev, and Angelidaki 2010; Taniguchi et al. 2005).

Furthermore, the use of a thermal pretreatment in this study was applied to ensure that only microorganisms that originated from the compost would be able to grow during the biological treatment. No positive effect was observed if only hydrothermal pretreatments (SWa) were applied to wheat straw samples. It is known that mild reaction temperatures result in incomplete reactions and no disruption of lignocellulose structure can be achieved (Chandra, Takeuchi, and Hasegawa 2012). In contrast, the use of LD as an additive for thermal hydrolysis had a positive effect (SDa) on decomposing lignocellulose components in straw.

The addition of compost and LD to wheat straw showed a synergistic effect on the degradation of the lignocellulosic fibers of pretreated samples. Cellulose and hemicellulose degradation were higher under thermophile anaerobic environments (SCWa-B and SCDa-B) than in aerobic conditions (SCWa-I and SCDa-I). It seems that the use of compost as an additional pretreatment ensured the growth of thermophile cellulose-degrading anaerobic microorganisms. The degree of degradation of cellulose and hemicellulose of the pretreated samples under thermophile anaerobic conditions ranged between 25 – 44% and 17 – 38%, respectively (Figure 14).

Furthermore, inhibited substances can be formed if thermal pretreatment is applied to lignocellulose feedstocks (Crespo et al. 2012). It seems that neither furfural nor hydroxymethyl furfural (HMF) could be observed in this study due to the mild reaction conditions during thermal pretreatment (Chandra, Takeuchi, and Hasegawa 2012).

4.4.3 BMP Determination

The methane potential of untreated straw as well as the pretreated samples (SW, SD, SWa, SDa, SCWa-I, SCDa-I, SCWa-B, and SCDa-B) was investigated as laboratory-scale batch tests at 37 °C. Results are depicted in Figure 15 and Figure 16.

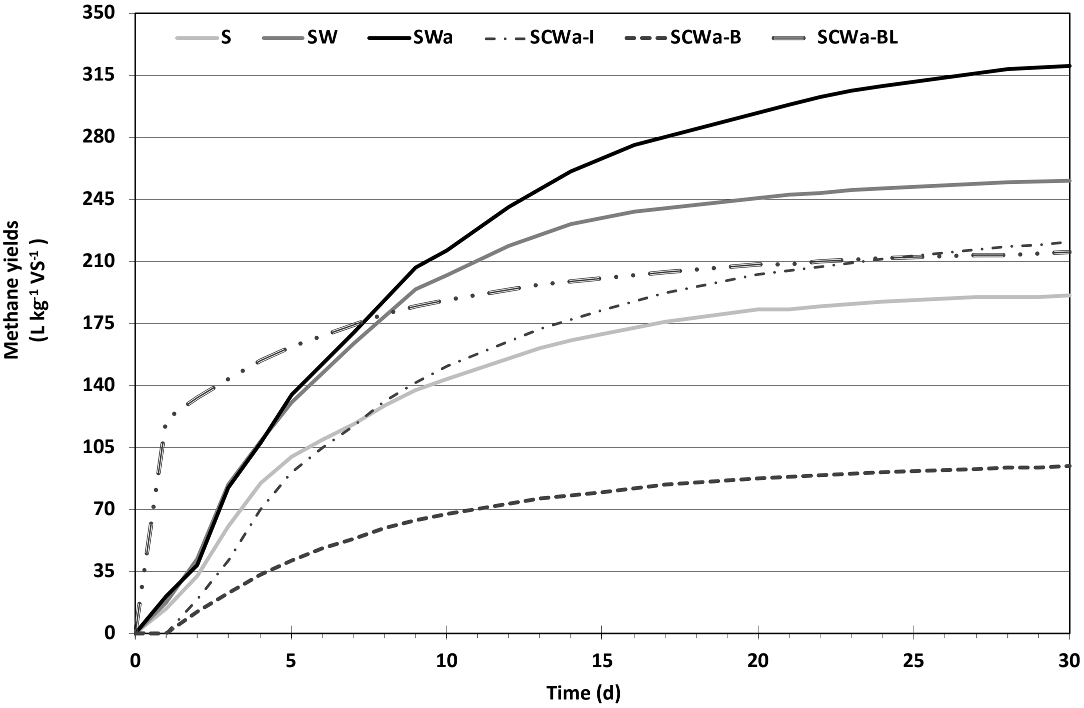

Figure 15: Cumulative methane yields (L kg^{-1} VS^{-1}) for samples SW, SWa, SCWa-I and SCWa-B; SW – straw with water; SCW – straw with compost and water; a – autoclaved; I – aerobically incubated for 14 days at 25 °C; B – anaerobically incubated for 14 days at 55 °C; BL: anaerobic incubation.

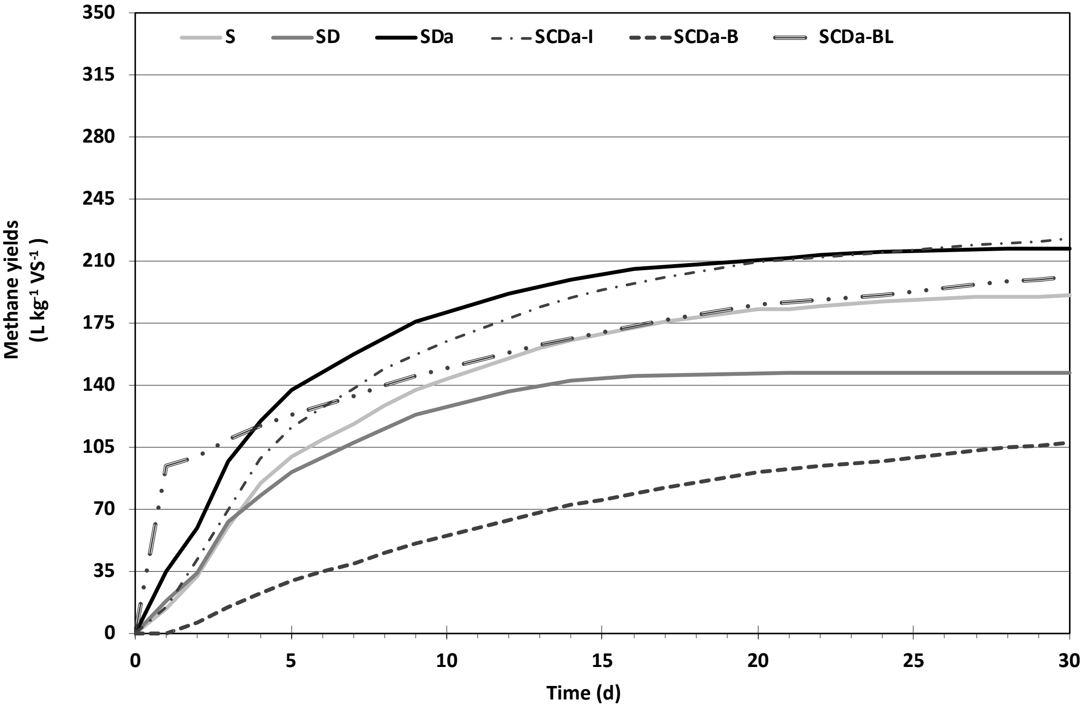

Figure 16: Cumulative methane yields (L kg^{-1} VS^{-1}) for samples SD, SDa, SCDa-I and SCDa-B; SD – straw with liquid digestate; SCD – straw with compost and liquid digestate; a – autoclaved; I – aerobically incubated for 14 days at 25 °C; B – anaerobically incubated for 14 days at 55 °C; BL: anaerobic incubation.

The curves from SCWa-BL (Figure 15) and SCDa-BL (Figure 16) show the methane that was produced and then measured during the anaerobic incubation and the methane yields from SCWa-B (Figure 15) and SCDa-B (Figure 16), respectively. Taking the methane produced before the batch test was run, i.e., prior to the AD of the B samples into account, the overall methane yields ranged between 190 and 320 L kg^{-1} VS^{-1} (Figure 15 and Figure 16). Methane production from digested straw obtained in this attempt was lower compared to those previously reported (Reinhold and Friedrich 2012; Kaparaju et al. 2009; Lehmann 2016).

In a previous study carried out by the authors, batch fermentation tests resulted in 261 L -kg^{-1} VS^{-1} (Bursche et al. 2018). The DM and VS content of feedstock used in those tests by Bursche et al. (2018) differed from the wheat straw contents used in this study. Therefore, the results suggest that differences in the DM and VS content in the straw used in both trials modified the methane potential of wheat straw. Methane production occurred immediately in most samples whereas a lag phase was observed in those that were previously pretreated with compost. The high amounts of lignocellulosic material found in SCWa-I, SCDa-I, SCWa-B, and SCDa-B pretreatment samples, probably accounted for the lower methane production. Anaerobic digestion from the thermally pretreated sample (SWa) showed the highest methane potential among the pretreatments. Literature on thermal pretreatment at 120 °C also showed an increase of at least 20% on biogas yields when digesting straw (Rajput and Sheikh 2019; Bolado-Rodríguez et al. 2016; Ferreira et al. 2013). Thus, thermally pretreating the substrate can increase the availability of fermentation products to the biomethanization (Fachagentur Nachwachsende Rohstoffe e.V. 2016; Ferreira et al. 2013).

Moreover, decreasing the DM content of biomass to 30% was suitable to further promote the AD using wheat straw as a substrate. Digesting SW samples resulted in higher methane yields than untreated straw (254 L kg^{-1} VS^{-1} to SW and 190 L kg^{-1} VS^{-1} to S). A suitable DM content of biomass is necessary to promote microorganism growth and to avoid the availability of high concentrated inhibiting substances due to the lower water content (Fachagentur Nachwachsende Rohstoffe e.V. 2016).

During incubation, additional methane formation of about 120 L kg^{-1} VS^{-1} (SCWa-BL) was observed in SCWa-B and 94 L kg^{-1} VS^{-1} (SCDa-BL) in SCDa-B. If the SC mixture was anaerobically pretreated, such an applied biological pretreatment promoted the degradation of the lignocellulose material, which stimulated biomethanization during incubation. Methane yields resulted in approximately 221 L kg^{-1} VS^{-1} and 222 L kg^{-1} VS^{-1} for SCWa-I and SCDa-I, respectively.

Although the biomethanization of these samples resulted in approximately 16% more methane than in untreated straw samples, only the organic matter loaded to the batch tests should be considered here. For this purpose, a correlated methane production assuming only the amounts of straw added to mixture was assessed. The correlated methane production took only the organic matter of wheat straw loaded to the biomethanization into account. Hence, correlated methane levels of the pretreated samples SCWa-

I, SCDa-I, SCWa-B(L) and SCDWa-B(L) were higher than the measured. The correlated methane production of all samples is shown in Figure 17.

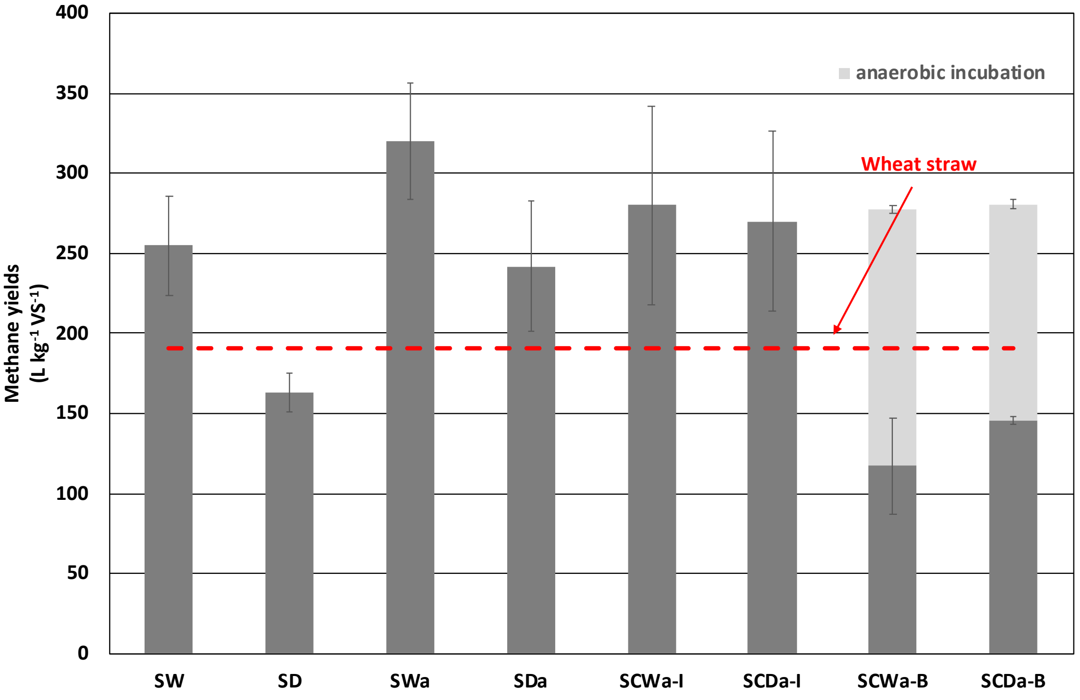

Figure 17: Specific methane yields from wheat straw and pretreated samples; SW – straw with water; SD – straw with liquid digestate; C – sample with compost. a – autoclaved; I – aerobically incubated for 14 days at 25 °C; B – anaerobically incubated for 14 days at 55 °C.

With the exception of SD, SCWa-B and SDWa-B, the correlated methane yield of all samples seemed to produce higher methane levels than wheat straw. However, SCWa-B and SCDa-B were first anaerobically incubated under thermophilic conditions and the additional methane formation of 160 L kg^{-1} VS^{-1} and 121 L kg^{-1} VS^{-1} for SCWa-BL and SCDa-BL, respectively, should be considered here.

A synergetic effect with an enhancement of up to 20% was observed in the methane production when samples were pretreated thermally. Results were in accordance with previous work, which also investigated the effects of thermal pretreatments to optimize the AD of straw. Methane production, which ranged between 270 – 350 L kg^{-1} VS^{-1}, was measured after pretreating the feedstock thermally (Yadav et al. 2019; Bauer et al. 2009; Mustafa et al. 2017; Chandra, Takeuchi, and Hasegawa 2012). Moreover, a steam explosion pretreatment with higher temperatures accounted for 350 L kg^{-1} VS^{-1} methane production (Rajput and Sheikh 2019; Bolado-Rodríguez et al. 2016; Ferreira et al. 2013; Bauer et al. 2009). Nevertheless, the amounts of energy required to pretreat the lignocellulosic feedstock thermally can counteract its use (Yadav et al. 2019; Frigon, Mehta, and Guiot 2012).

Methane production of SWa was higher than SCWa-I, despite the fact that an additional biological pretreatment was applied to SCWa-I. As already known, under aerobic conditions, biological pretreatment can stimulate the growth of microorganisms such as yeast and molds leading to a reduction in valuable carbon sources in the substrate to be digested. Coupling the addition of aerobic microorganism with a post-anaerobic fermentation as a biological pretreatment, showed no improvement in methane production regarding microbiological pretreatment of wheat straw samples (Thomsen et al. 2016).

There was a paradigm shift in the degradation of the lignocellulose material of samples pretreated with LD and methane production considering that during pretreatment degradation of structural carbohydrates took place (Figure 14). Even though the applied biological pretreatment would optimize the use of feedstock for AD due to the higher concentration of soluble components, it seems that an inhibition process could occurred and further improvement on biomethanization could not be observed. In accordance with this, Hu et al. (2014) and J. Sun et al. (2019) reported that the pretreatment time and the particle size of the substrate are two crucial parameters in the chemical pretreatment with LD, i.e., these parameters influence the ability of the substrate to interact efficiently with the LD. In Hu et al. (2014), for example, a decrease in methane production was observed after 5 days of pretreatment time. For this reason, there are doubts about a positive effect of adding compost to straw if the substrate is inoculated with LD for more than 14 days. Sun et al. (2019) determined that the particle size had no obvious influence on the performance of the anaerobic methanization if the pretreatment time was over to 5 days.

The use of compost as a natural source of microorganisms that are able to degrade lignocellulose feedstocks showed a favorable effect on methane production in samples that had been previously anaerobically incubated. In accordance with this, the use of compost showed a positive effect when using compost as an inoculum in batch fermentation tests (Neumann and Scherer 2011). The addition of compost resulted in faster biogas production and an increase of 6% at mesophilic conditions (Neumann and Scherer 2011).

Although SCWa-B and SCDa-B showed the lowest correlated methane production (117 L kg^{-1} VS^{-1} and 146 L kg^{-1} VS^{-1}), the fermentation process already started during incubation before AD. Methane production of SCWa-BL started immediately during the incubation phase and reached about 84% of the methane yield of untreated straw after 14 days of incubation.

Therefore, the technical digestion time of samples was assessed to evaluate the efficiency of the process (Table 11). The technical digestion time (T_{80}) is defined as the time required to produce 80% of the maximum biogas volume. A small value of T_{80} means high efficiency at low cost (Kim and Lee 2005). Considering this, T_{80} is related to 80% of the total methane yield of wheat straw and pretreatments.

Table 11: Comparison of the technical digestion time of wheat straw with pretreated samples, based on the amount of straw contained (no data for SD, due to the fact that the total methane yield is less than S).

Parameters	**S**	**SW**	**SWa**	**SDa**	**SCWa-I**	**SCDa-I**	**SCWa-B**	**SCDa-B**
T_{80} Methane yield (L kg^{-1} VS^{-1})	152.59	146.95	152.36	147.54	148.81	155.60	163.05	153.67
T_{80} (days)	11.5	7	6	8	7.5	6	1	3
shorter digestion time (%)	-	39	48	30	35	52	91	74

The T_{80} values indicated that the digestion time of samples pretreated with compost were shortened by 35% – 91% compared to the untreated wheat straw fermentation. More than 84% of methane production from wheat straw was already formed during biological pretreatment of SCWa-B, suggesting that pretreating wheat straw with compost has great potential to improve anaerobic fermentation efficiency.

4.5 Conclusion

The effect adding green waste compost as a biological pretreatment to improve the biogenic catalysis of wheat straw to enhance the use of lignocellulose biomasses for a biorefinery concept was investigated in this study. For this purpose, a thermo-biological pretreatment was carried out using a mixture of mechanically comminuted wheat straw and green waste compost as a substrate. Dry matter content of samples was adjusted using either water or liquid digestate prior pretreatments. Results revealed a lignocellulose degradation in all samples. There are doubts about a positive effect of adding compost to straw if the substrate is inoculated with LD for more than 14 days. In this respect, the influence of LD as a pretreatment to improve the AD of wheat straw should be further investigated. The use of compost ensured the growth of cellulose-degrading anaerobic microorganisms under thermophilic conditions. Due to the sugar compounds released during the biogenic catalysis, the hydrolysis process – which is considered the rate-limiting step in anaerobic digestion – was improved. Moreover, further studies are needed to link the chemical composition and dynamics of microbial diversity during incubation using molecular biological methods to elucidate the role of microorganisms and to better understand their mechanisms during incubation in anaerobic digestion.

4.6 Acknowledgment

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Commission and the European Regional Development Fund (ERDF) under the slogan “Investing in our future”.

4.7 Symbols used

Symbols

T_{80}	[days] technical digestion time

Abbreviations

AD	Anaerobic digestion
DM	Dry matter
LD	Liquid digestate
LTD	Limited
S	Wheat Straw
SCDa	Wheat Straw with liquid digestate and compost, autoclaved
SCDa-B	Wheat Straw with liquid digestate and compost, autoclaved, anaerobically incubated for 14 days at 55 °C
SCDa-BL	Wheat Straw with liquid digestate and compost, autoclaved, methane yield during anaerobic incubation for 14 days at 55 °C
SCDa-I	Wheat Straw with liquid digestate and compost, autoclaved, aerobically incubated for 14 days at 25 °C
SCWa	Wheat Straw with water and compost, autoclaved
SCWa-B	Wheat Straw with liquid digestate and compost, autoclaved, anaerobically incubated for 14 days at 55 °C
SCWa-BL	Wheat Straw with water and compost, autoclaved, methane yield during anaerobic incubation for 14 days at 55 °C
SCWa-I	Wheat Straw with water and compost, autoclaved, aerobically incubated for 14 days at 25 °C
SD	Wheat Straw with liquid digestate
SDa	Wheat Straw with liquid digestate, autoclaved
SW	Wheat Straw with water
SWa	Wheat Straw with water, autoclaved
VS	Volatile solids
W	Water

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5 Effects of thermo-biological pretreatments on the combustion properties of wheat straw in a cascaded biorefinery concept

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Notes on the supplementary data for this chapter can be found in the Appendix.

5.1 Abstract

In this study, the effects of different thermo-biological pretreatments in combination with a washing process on the combustion properties of wheat straw were investigated in order to optimize its use as solid fuel. An essential part of the biological pretreatment for fermentative applications is the use of green waste compost. The compost provides the necessary microorganism to break down the lignocellulose contained in the straw and as consequence influence the combustion properties as well as making the dissolved sugar polymers usable. However, the use of compost as an additive in biomass combustion applications has been scarcely studied. In the following analysis, the effects on ash content, calorific value, and ash melting behavior were verified and an analysis of variance (ANOVA) was performed. The results show that thermal treatment by autoclave with biological treatment by anaerobic incubation in combination with the washing process are promising pretreatment procedures for wheat straw for pellet production, improving the above-mentioned combustion characteristics of the straw and hence meeting the requirements of ISO 17225-6 for non-woody pellets. For the straw–compost mixtures, the applied combined pretreatment is a possible solution to the problems related to low ash melting temperatures, as the shrinkage temperatures were increased over 659 °C compared to raw straw. Adjustments in process design have to be made as well as the compost content has to be reduced to ensure that the ash content as well as the calorific value meet the requirements of ISO 17225-6. The potential of compost is promising and can be considered as a possible additive for pellet production from wheat straw. This study also showed that the cascading use of straw–compost mixtures increases the value-added potential of a biorefinery, since solid fuels with optimized combustion properties can be provided in addition to liquid or gaseous fuels.

Keywords: Wheat straw, Compost, Pellets, Ash melting, Thermo-biological pretreatment, Biorefinery

5.2 Introduction

The future belongs to the circular economy (CE), which is mostly associated with an economic system that replaces the ‘‘end-of-life’’ concept and aims to increase the efficiency of resource use, focusing on agricultural, municipal, and industrial residues (Bezama et al. 2019; Brosowski et al. 2019; Kirchherr, Reike, and Hekkert 2017; Murray, Skene, and Haynes 2017; Sodhi et al. 2022). The goal is to accomplish sustainable development, thus simultaneously creating environmental quality, economic prosperity, and social equity, to the benefit of current and future generations (Kirchherr, Reike, and Hekkert 2017). The traditional linear economy of ‘‘take, make, waste’’ is not compatible with current global challenges such as climate change, population growth, and unsustainable lifestyles (Murray, Skene, and Haynes 2017; Kirchherr, Reike, and Hekkert 2017; Sodhi et al. 2022). Coordinated biorefineries are the backbone of CE when they utilize all components of biomass residue to produce energy, vital substances, and chemicals (Jain et al. 2022). The biogenic lignocellulosic residue wheat straw is an abundant feedstock for the production of bioproducts or bioenergy and is therefore gaining interest because it does not compete with food production, is affordable, and its use as a feedstock supports the shift toward a circular economy (da Silva, Torres Ortega, and Rong 2016; Bursche, Rieker, and Beuel 2019; Beuel, Rieker, and Bursche 2019; 2020). The worldwide production of wheat in 2020 was estimated at 775 million tons, which leaves 354 million tons of wheat straw available for the generation of bioproducts and bioenergy (Ingrao et al. 2021). Wheat Straw consists mainly of cellulose (30% – 50%), hemicellulose (20% – 35%), and lignin (15% – 20%) (Bursche, Rieker, and Beuel 2019; Beuel, Rieker, and Bursche 2020).

To optimize biodegradability and fully exploit the energetic potential of lignocellulosic biomass, to produce second-generation biofuels for instance, pretreatment is required to increase the formation of water-soluble sugars by delignification and degradation of cellulose and hemicellulose (da Silva, Torres Ortega, and Rong 2016; Bursche, Rieker, and Beuel 2019; Beuel, Rieker, and Bursche 2020). Pretreatments can take place as physical, chemical, biological or a combination of them and due to the wide variety of these materials, it is difficult to find a general process design or to define the optimal pretreatment method for a biorefinery (Bursche, Rieker, and Beuel 2019; Galbe and Wallberg 2019). Considering the idea of CE, it is ultimately linked to the resource cycle, therefore pretreatments which integrates the biogeochemical cycle are of particular interest (Murray, Skene, and Haynes 2017; Jain et al. 2022). Bursche et al. (2019) investigated the use of compost as a natural source of microorganisms (e.g. bacteria and fungi) to degrade structural carbohydrates from lignocellulosic wheat straw after thermal treatment in an autoclave as a combined pretreatment approach. To further explore the observed paradigm shift in the study of Bursche et al. (2019), the authors of this present study investigated those concepts and the effect of adding green waste compost as a biological pretreatment for improving the bioconversion of wheat straw to enhance the use of lignocellulose biomasses for a biorefinery concept (Beuel, Rieker, and Bursche 2020). An adapted thermo-biological pretreatment was carried out and the results showed that lignocellulose was degraded in all samples, resulting in increased methane production with reduced retention time (Beuel, Rieker, and Bursche 2020).

Besides the restricted biodegradability of straw, it is well-known by its inferior combustion properties such as low calorific value (CV), high ash content, and low melting temperature of ashes, which makes it difficult to use as a solid fuel (Toscano et al. 2019; Vassilev et al. 2017; 2013). This can cause slagging, fouling and in some cases corrosion of boilers, which reduces the efficiency of combustion systems (Toscano et al. 2019; Vassilev et al. 2017; Nosek et al. 2020; Vassilev et al. 2013). According to the studies of Saddawi et al. (2012), Stelte et al. (2013), Azócar et al. (2019), Scherzinger et al. (2020), and Cheng et al. (2022), thermal pretreatment methods such as autoclaving, torrefaction, and hydrothermal carbonization have been successfully applied to improve the overall fuel properties of solid biofuels, while the leaching effect of minerals by hydrothermal carbonization of rape straw has also been reported in Cheng et al. (2022).

However, in order to enhance the combustion properties and especially the melting temperatures, the use of additives has recently been proposed in the literature (Wu et al. 2011; Wang et al. 2011; Matúš et al. 2018; Toscano et al. 2019; Nosek et al. 2020). Various waste materials, especially those of natural origin, are of particular interest as additives (Wang et al. 2011; Nosek et al. 2020; Matúš et al. 2018). To the best of our knowledge, compost has not yet been considered as an additive. In a review about the utilization of compost Chia et al. (2020) reported that various studies have produced solid fuels from compost and some of these studies used materials such as coal tailings, sawdust or wood chippings as an additive in order to reduce ash content and to increase the calorific value. In an extended review on the composting of green waste, Reyes-Torres et al. (2018) indicated that despite the reported heterogeneity, green waste is characterized by low contents of alkali metals such as potassium, which favor low sintering and ash melting temperatures. Besides, the study reported contents of alkaline earth metals like calcium and magnesium. Calcium and magnesium normally increase the melting temperature of ashes (Vassilev et al. 2017). The silicon bonded in wheat straw will react with calcium with formation of calcium silicates that have higher melting temperatures than potassium silicates (Vassilev et al. 2013; 2017). Furthermore, magnesium respectively magnesium oxide prevents the sintering of ash up to 1,100 °C, forms higher melting compounds and binds sulfate, resulting in magnesium sulfate with a melting point above 1,124 °C (Toscano et al. 2019). It is therefore expected that the use of green waste compost will increase the ash melting temperatures due to its chemical composition (Vassilev et al. 2013; 2017; Reyes-Torres et al. 2018; Toscano et al. 2019). Regarding the ash content that compost could provide, the literature reported varying values between 40 – 65% (Zajonc, Frydrych, and Jezerska 2014; Kliopova and Makarskienė 2013). However, ash content below 7% was also found (Beuel, Rieker, and Bursche 2020).

Interestingly, different studies have also investigated the effect of washing wheat straw with water as a solvent, as this can remove minerals that reduce the melting temperature of the ash and nevertheless significantly reduce the ash content (Gudka et al. 2016; Saddawi et al. 2012; Deng, Zhang, and Che 2013; Singhal, Konttinen, and Joronen 2021a; 2021b). The use of water as a solvent during the washing process providing an alternative and additional method to improve the combustion properties of wheat straw as well as wheat straw–compost mixtures (Gudka et al. 2016; Weiß and Glasner 2018). Moreover, during this process hydrophilic sugar components dissolve and pass into the liquid. The subsequent separation of the liquid and solid phases also results in the possibility of using these two material streams separately in a biorefinery concept, e.g., fermentation of the liquid phase and processing of the solids into fuels. Nevertheless, the above-mentioned possibilities to improve the combustion properties of wheat straw have not yet been integrated into a cascaded biorefinery concept. This research combines different thermal pretreatments together with the leaching effects of a washing process to study the impacts and consequently attempt to improve the combustion properties of wheat straw. It also proposes to analyze the influence of compost as an additive for a solid fuel based on wheat straw, taking into consideration the promising results of compost in conjunction with wheat straw as part of a thermo-biological pretreatment in fermentative processes for methane production (Bursche, Rieker, and Beuel 2019; Beuel, Rieker, and Bursche 2020).

Therefore, this study defines two main objectives: (i) to investigate the combustion properties of wheat straw after thermo-biological pretreatment, (ii) to investigate the suitability of compost as an effective additive regarding chemical composition and to study its effects on the combustion properties of wheat straw–compost mixtures. For this purpose, different thermo-biological pretreatments for wheat straw and wheat straw–compost mixtures were applied and their impacts on combustion properties, such as ash content, calorific value, and ash melting behavior evaluated. Considering the advantages not only in terms of combustion properties, but also for further value-added opportunities of a biorefinery, an experimental washing process was performed as part of this research.

5.3 Material and methods

5.3.1 Raw materials

Wheat straw (straw) was obtained from a horse farm close to Cologne in Germany. Green waste compost (compost) used during the biological pretreatment was obtained from the green waste composting plant in the *Leppe* waste disposal center in Lindlar. The "Bergischer Kompost" used has been awarded the RAL quality mark of the *Bundesgütegemeinschaft Kompost e.V.* (AVEA GmbH & Co. KG. 2021; Bundesgütegemeinschaft Kompost e.V. 2021). The composition of used substrates is shown in Table 12.

Table 12: Composition of used substrates.

Parameter	**Straw**	**Compost**
DM^{a} [%]	92.63 ± 0.4	44.34 ± 4.69
VS^{a} [%]	95.37 ± 2.27	49.20 ± 9.01
Cellulose [% DM]	48.85 ± 1.37	14.03 ± 0.59
Hemicellulose [% DM]	29.06 ± 1.79	3.38 ± 0.46
Klason Lignin [% DM]	16.06 ± 4.69	69.10 ± 2.47

^{a} Dry matter [DM], volatile solids [VS].
^{b} After sieving.

5.3.2 Mechanical and thermo-biological pretreatment

The sequence of the thermo-biological pretreatment process for straw–water (SW) and straw–compost (SCW) based samples is shown in Figure 18.

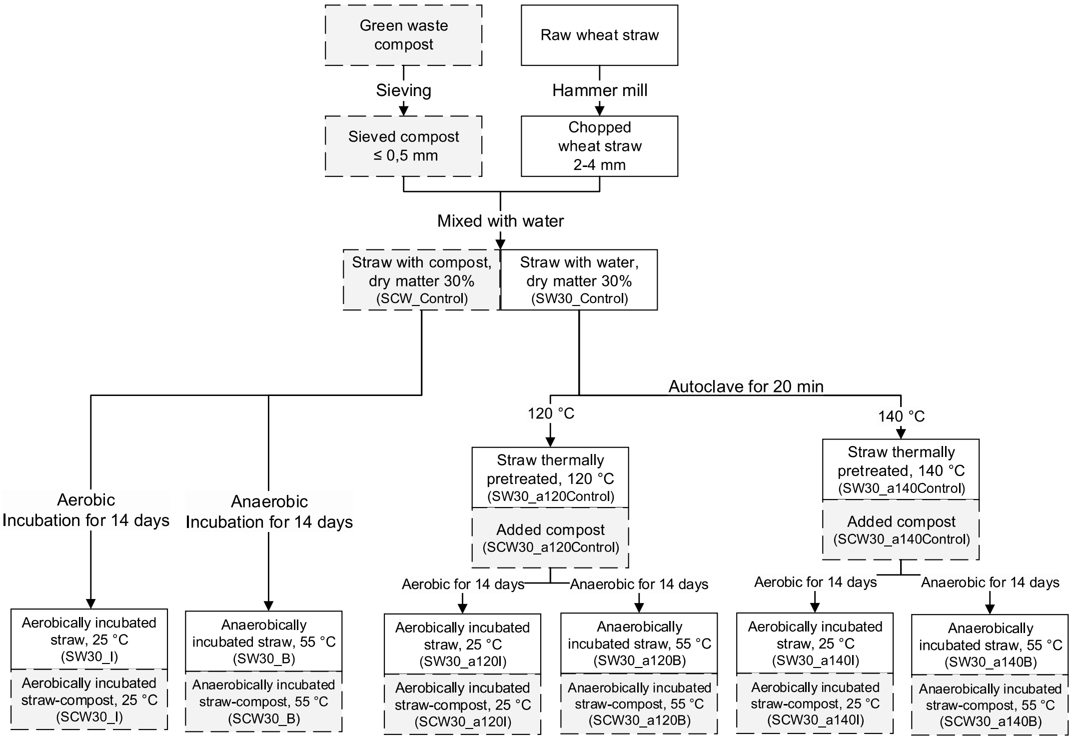

Figure 18: Sequence of thermo-biological pretreatment.

For the preparation of SW as well as SCW samples, raw straw was chopped to a particle size of 2 – 4 mm using a hammermill with 22 kW (*Mütek Systemtechnik*, Type MPZ 600).

The compost was sieved to remove large fractions of material using a sieve shaker (*Retsch*, AS 400). The chopped straw was mixed with water to obtain a dry matter (DM) of 30% (SW30_Control); sieved green waste compost (Compost) was mixed with straw to a homogeneous mixture with a DM of 30% (SCW30_Control). Then the samples were incubated for 14 days under aerobic conditions at 25 °C (SW30_I, SCW30_I) and anaerobic conditions at 55 °C (SW30_B, SCW30_B). In parallel, the SW30_Control sample was thermally pretreated in an autoclave for 20 minutes at 120 °C (SW30_a120Control) and at 140 °C (SW30_a140Control); then compost was added for SCW Samples (SCW30_a120Control, SCW30_a140Control). Next, these two samples were pretreated for 14 days under aerobic incubation at 25 °C (SW30_a120I, SW30_a140I and SCW30_a120I, SCW30_a140I), as well as an anaerobic incubation at 55 °C (SW30_a120B, SW30_a140B and SCW30_a120B, SCW30_a140B). The prepared straw–water and straw–compost mixture samples and their designations and associated descriptions are summarized in the supplementary data in Table S1 (see Appendix).

5.3.3 Preparation of samples and analytical framework

The workflow scheme in Figure 19 provides an orientation for the individual analysis steps of this study. All analyses were carried out in accordance with current ISO methods for the characterization of solid biofuels and are briefly summarized in the following sections.

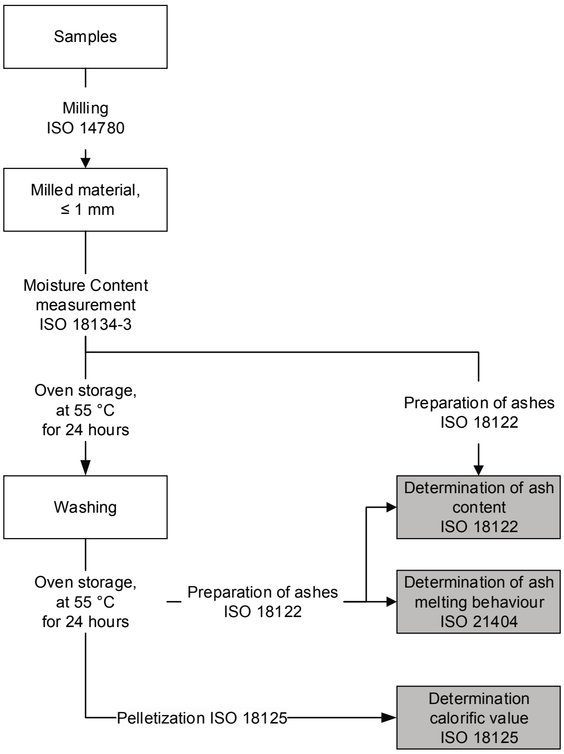

Figure 19: Workflow scheme for the analysis of combustion properties of pretreated SW and SCW samples.

5.3.3.1 Milling and moisture content

The samples were prepared following ISO 14780; therefore, an Ultra Centrifugal Mill (*Retsch*, ZM 200) was used. All the materials were milled and had a nominal particle size of 1 mm or less, except for the sieved compost, as it had already a particle size of less than 1 mm. The samples were dried in a laboratory oven at 105 °C in air atmosphere for 24 h until a constant mass was achieved, following the guideline ISO 18134-3.

5.3.3.2 Ash content

According to the procedure described in ISO 18122, the ash content on dry basis (d.b.) of each sample was determined at 550 °C in an automatic muffle furnace (*Nabertherm*, LE 4/11/R6).

5.3.3.3 Gross calorific value (GCV) and net calorific value (NCV)

The guideline ISO 18125 was followed to determine the GCV. As a test portion, a pellet of mass (1.0 ± 0.2) g was pressed with a suitable force to produce a compact, unbreakable test piece. To achieve this, a hand-operated press was used (IKA, C21). Once obtained, the pellet was tested in the calorimeter (IKA, C200). To calculate the NCV, the formula provided in ISO 18125 was used. For the samples investigated and according to the literature reviewed, a straw hydrogen content value of 5.5% on dry basis was used (Matúš et al. 2018; Gudka et al. 2016; University of Technology Vienna and Institute of Chemical Engineering 2021; Loo and Koppejan 2008; Obernberger and Thek 2010). Moisture content is obtained as already explained in 5.3.3.1.

5.3.3.4 Ash melting behavior

The method of determination of ash melting behavior is followed according to ISO 21404. Furthermore, the guide defines four characteristic temperatures that describe the tendency of ashes to form deposits or slagging in the ember layer during heating: Shrinkage starting temperature (SST), Deformation temperature (DT), Hemisphere temperature (HT), Flow temperature (FT). The thermo-optical analysis was conducted using a Heating Microscope (*Hesse*, EM301). The outer shape changes of each sample were recorded as the temperature increased from 550 to 1,500 °C. The ash used for the test was a homogeneous material, prepared from the fuel by ashing at 550 °C as explained in 5.3.3.2 according to the guideline ISO 18122.

5.3.3.5 Washing process

Distilled water was used as a solvent for the washing process. Each material, except for compost, was placed in a full-page blender bag with a full surface micro-perforated filter (*Interscience by Interlab*) and then distilled water was added. Following recent studies on washing processes, this study established a solid-to-liquid (S:L) ratio of 1:25, which favors the removal of Cl, S, ash, and N compared to lower S:L ratios (Gudka et al. 2016; Deng, Zhang, and Che 2013; Singhal, Konttinen, and Joronen 2021a). Each mixture was then homogenized for 4 minutes at 8 strokes per second using a Masticator with a dual paddle system (IUL Instruments, Silver) to efficiently mix the samples and avoid the risk of cross-contamination. After homogenization, the liquid was manually separated from the solid using a filter (0.2 µm pore-size membrane filter, *Interscience by Interlab*) and funnel. Afterwards, the process was continued by centrifugation (12,500 rpm for 15 minutes; *Hermle*, Z 327 K).

5.3.3.6 Statistical analysis

To evaluate the effects of the pretreatments on the combustion results, an analysis of variance (ANOVA) was performed. The pretreatment carried out in this study was performed with three types of incubation (without incubation, aerobic at 25 °C and anaerobic at 55 °C) and different autoclave temperatures (without autoclave, at 120 and 140 °C). Thus, the factors are (1) the type of incubation and (2) the autoclave temperatures. Hence, a two-factor analysis of variance was performed considering the information obtained from the samples with a p-value ≤ 0.05. If the p-value ≤ 0.05, it was concluded that the effect being tested is statistically significant (Hinkelmann and Kempthorne 2012). The two-sample t-test was used to determine if two population means are equal. A null hypothesis $H0 : \mu1 \leq \mu2$ was established, the alternative hypothesis $H1 : \mu1 > \mu2$ was considered as a two-sided hypotheses test. An $\alpha = 0.025$ was considered according to the literature (Hinkelmann and Kempthorne 2012). Results, for both analysis of variance and two-sample t-test (shown in supplementary material), were calculated with *Microsoft Excel*, using information obtained from the samples.

5.4 Results and discussion

5.4.1 Ash content

The ash content results for the pretreated SW and SCW samples before and after the washing process are presented in Table 13. Untreated straw has an ash content average of 6.2%. This value is in accordance within the values found in the literature for straw (Beuel, Rieker, and Bursche 2020; Azócar et al. 2019; Matúš et al. 2018; Deng, Zhang, and Che 2013). The compost sample has the highest ash content of 69% and is comparable to the values reported in the literature (Zajonc, Frydrych, and Jezerska 2014; Kliopova and Makarskienė 2013). All unwashed pretreated SW samples showed a decrease in ash content after the pretreatments compared to untreated straw. Comparing the ash content between compost and unwashed pretreated SCW samples, the latter have also a lower ash content. A large variability is observed in the unwashed pretreated SCW samples (Table 13).

Table 13: Average ash content (% d.b.) of pretreated samples before and after the washing process, untreated straw and untreated compost.

Code	Samples	Unwashed	Washed
SW_1	SW30_Control	5.8	2.5
SW_2	SW30_I	6.1	1.6
SW_3	SW30_B	5.5	2.4
SW_4	SW30_a120Control	6.1	3.9
SW_5	SW30_a120I	5.7	3.5
SW_6	SW30_a120B	5.9	0.5
SW_7	SW30_a140Control	5.7	4.0
SW_8	SW30_a140I	5.7	2.8
SW_9	SW30_a140B	5.3	2.5
SCW_1	SCW30_Control	43.7	22.2
SCW_2	SCW30_I	38.1	22.5
SCW_3	SCW30_B	43.3	25.4
SCW_4	SCW30_a120Control	39.9	24.3
SCW_5	SCW30_a120I	48.5	23.9
SCW_6	SCW30_a120B	43.3	24.9
SCW_7	SCW30_a140Control	34.0	22.8
SCW_8	SCW30_a140I	33.6	12.9
SCW_9	SCW30_a140B	27.5	22.3
S	Straw (untreated)	6.2	tbd
C	Compost (untreated)	69.0	tbd

ANOVA was used to determine the significance of the pretreatments for the pretreated SW (Appendix, Table S2) and SCW samples (Appendix, Table S4). The thermal pretreatment of SW samples using an autoclave at 120 °C or 140 °C had a significant effect and decreased the ash content. In comparison to other thermal treatments, i.e., torrefaction, different studies observed a low ash increment of up to 17% under light torrefaction conditions at 145 °C and 150 °C, respectively (Azócar et al. 2019; Stelte et al. 2013). Biological pretreatment by incubation, either aerobically at 25 °C or anaerobically at 55 °C, also significantly influenced and decreased the ash content. The microorganisms involved in the degradation of lignocellulose during aerobic and anaerobic incubation probably utilize the minerals contained in the ash, such as potassium (K), chlorine (Cl), sulfur (S) or sodium (Na), thus also reducing the ash content (Bursche, Rieker, and Beuel 2019; Beuel, Rieker, and Bursche 2020; Romero-Güiza et al. 2016). The interaction of both pretreatments proved to be significant in all combination (Table 13, Appendix Table S2) and led to a decrease of ash content. As expected, the washing process leads to a further reduction in ash content (Table 13), as various minerals that promote the formation of ash (i.e., K, Cl, S or Na) were removed (Gudka et al. 2016; Saddawi et al. 2012; Deng, Zhang, and Che 2013; Singhal, Konttinen, and Joronen 2021b; 2021a). To evaluate this hypothesis, a t-test analysis was used (Appendix, Table S3). It can be concluded that for the SW pretreated samples, all separately applied or combined pretreatments significantly affected the ash content. In comparison to raw straw, an average reduction of 5.8% after pretreatment and 55.8% after pretreatment and washing was observed.

In contrast to SW samples, only the thermal pretreatment at either 120 °C or 140 °C significantly affected the ash content of SCW samples (Appendix, Table S4). As recommended in the literature a new ANOVA (Appendix, Table S5) was performed excluding the interaction variable (Hinkelmann and Kempthorne 2012). Results showed that the use of the autoclave was the only variable that had a statistically significant effect on the ash content of the SCW samples (Appendix, Table S5). In this respect, the unwashed pretreated SCW samples subjected to autoclave treatment at 140 °C had the lowest ash content of the nine samples. Compared to SCW_1 without thermal treatment at 140 °C, ash reductions of 37%, 23%, and 22%, respectively, were observed. This corresponds to an average reduction of 27.3%. Therefore, better results were obtained when pretreatment was performed in an autoclave at 140 °C. Although no statistical significance was determined for the incubation of the SCW samples, in comparison with the SW samples it can be observed that the effect of biological pretreatment with compost in combination with thermal treatment led to a greater reduction in ash content. This is probably due to the microorganisms introduced, which led to a greater degradation of lignocellulose in combination with thermal treatment and thus lower ash content (Theuretzbacher et al. 2015; Scherzinger, Kulbeik, and Kaltschmitt 2020).

Meanwhile, for the washed pretreated SCW samples (Table 13), the ash content stabilizes at 22% – 25%, except for sample SCW_8 (13%, Table 13). Similar to the SW samples, t-test analysis (Appendix, Table S6) was used to confirm the effect of the washing processes. Pretreated and washed SCW samples revealed a strong decrease in ash content with an average of 22.4% ± 3.7% (Table 13).

After the washing process, an average ash reduction of 43% was achieved for the SCW and 55% for the SW samples. These results are consistent with conclusions in the literature (Gudka et al. 2016; Saddawi et al. 2012; Deng, Zhang, and Che 2013); the ash content decreases after washing with water and hence it can be assumed that there is a removal of K, S, and Cl (Deng, Zhang, and Che 2013). The washing efficiency of this study exceeds the reported maximum ash removal of 39.3% from Singdahl et al., which was achieved for 0.05 – 0.08 cm samples after 3 h of washing at ambient temperature with a solid-to-liquid ratio of 1:15 (Singhal, Konttinen, and Joronen 2021a; 2021b). For further investigations, the applied washing process during this study should be optimized considering the industrial requirements.

In conclusion, the SW samples have an ash content of less than 10%, which meets the requirements of ISO 17225-6 for residential, small commercial and public building applications. In contrast, the SCW samples homogeneously mixed with compost (50:50 ratio) do not meet the requirements of ISO 17225-6 due to the high ash content of the compost. To obtain values within the standard, the straw–compost ratio should be adjusted to 60:40, 70:30, 80:20, and 90:10 and further investigated.

5.4.2 Calorific values and moisture content

The calorific values of pellets based on SW and SCW samples after pretreatment and washing with their respective moisture content are listed in Table 14. The average GCV of untreated straw pellets (17.7 MJ kg^{-1}) is in the normal range for this type of material (Azócar et al. 2019; Demirbaş 2001; Gudka et al. 2016; Saddawi et al. 2012; Deng, Zhang, and Che 2013; Singhal, Konttinen, and Joronen 2021b; 2021a). In contrast, the compost pellet has the lowest GCV (4.7 MJ kg^{-1}) and NCV (2.1 MJ kg^{-1}), which is lower than the values from other studies. Zajonc et al. (2014) investigated seven compost samples from six composting plants that were produced, for example, from sewage sludge (NCV of 5.7 MJ kg^{-1}) or with wood chips (NCV of 12.1 MJ MJ kg^{-1}), with an average NCV of 8.6 MJ MJ kg^{-1}. However, Kliopova and Makarskienė (2013) analyzed pellets produced from stabilized sewage sludge (about 50%), municipal green waste (about 26%), and other biomass residues with an NCV of 3.8 MJ MJ kg^{-1}. According to Reyes-Torres et al. (2018), the low calorific value of the green waste compost measured in this present study is related to its variable physical composition, which depends on aspects such as vegetation type, climatic conditions, and collection strategies. This variability ensures that there is no consistent physicochemical property for this feedstock. The pretreated SW pellets also were within the normal GCV ranges for straw with an average of 17.6 ± 0.4 MJ kg^{-1} (Azócar et al. 2019; Demirbaş 2001; Gudka et al. 2016; Saddawi et al. 2012; Deng, Zhang, and Che 2013; Singhal, Konttinen, and Joronen 2021b; 2021a). In comparison, the GCV of lignite coal ranges between 10 – 19 MJ kg^{-1} (Stelte et al. 2013; Alfattani et al. 2021).

Table 14: Average gross calorific value and net calorific value (MJ kg−1), moisture content (MC) (% d.b).

Code	Samples	GCV	MC	NCV
SW_1	SW30_Control	17.9	3.0	16.2
SW_2	SW30_I	18.1	5.3	15.9
SW_3	SW30_B	17.5	6.1	15.3
SW_4	SW30_a120Control	17.8	4.0	15.9
SW_5	SW30_a120I	17.2	4.9	15.1
SW_6	SW30_a120B	17.5	5.3	15.4
SW_7	SW30_a140Control	17.5	3.7	15.7
SW_8	SW30_a140I	16.9	4.9	14.9
SW_9	SW30_a140B	18.2	5.2	16.0
SCW_1	SCW30_Control	15.3	3.3	12.3
SCW_2	SCW30_I	12.6	4.7	9.6
SCW_3	SCW30_B	14.0	4.2	11.0
SCW_4	SCW30_a120Control	14.3	3.8	11.2
SCW_5	SCW30_a120I	16.0	3.6	13.0
SCW_6	SCW30_a120B	16.7	3.5	13.7
SCW_7	SCW30_a140Control	16.8	2.0	14.0
SCW_8	SCW30_a140I	16.7	4.8	13.4
SCW_9	SCW30_a140B	15.5	4.5	12.4
S	Straw (untreated)	17.7	2.9	16.0
C	Compost (untreated)	4.7	2.3	2.1

As shown in Table 14, the disparity of GCVs in the pretreated SW sample pellets indicates that the pretreatments did not significantly affect the heating value. It is also noticeable that four SCW pellet samples (SCW_5–6, SCW_8–9), which were subjected to biological pretreatment along with autoclaving have higher GCV than pellets that were subjected to incubation only. Moreover, when considering the autoclave pretreatment solely, a better result was obtained with the autoclave at 140 °C than at 120 °C, because relatively more compounds with relatively low CV such as hemicellulose were removed with increasing temperature (Azócar et al. 2019; Stelte et al. 2013; Scherzinger, Kulbeik, and Kaltschmitt 2020). To evaluate the influence of the pretreatments on the reaction variable GCV, an ANOVA analysis was performed. It can be assumed that the pretreatments have no statistical influence on the GCV of SW Samples (Appendix, Table S7). Although the investigated pellets were washed, they showed no discernible difference in GCV compared to untreated straw, which is largely consistent with the literature reviewed, which reports little to no improvement (Vassilev et al. 2013; Deng, Zhang, and Che 2013; Singhal, Konttinen, and Joronen 2021a).

In contrast, when working with SCW pellets, both the use of the autoclave and the interaction between incubation and autoclave have an effect on the calorific value (Appendix, Table S8). When autoclaving (regardless of temperature) is used in conjunction with incubation (regardless of type), relatively similar results are obtained, generating an increase in GCV. On average, these four types of pellets (SCW_5–6, SCW_8–9) had a GCV of 16.2 MJ kg^{-1}, which is equivalent to a 6% improvement over SCW_1. The combination of biological and thermal treatment, with a kind of steam explosion effect as in an autoclave, probably resulted in a higher hemicellulose removal in the SCW samples (Scherzinger, Kulbeik, and Kaltschmitt 2020; Sindhu, Binod, and Pandey 2016). In addition, the use of an autoclave at 140 °C leads to better results than autoclaving at 120 °C, an improvement of about 10% over SCW_1. Using the autoclave at 120 °C appears to result in a reduction in GCV of about 8%. On the other hand, as described in Demirbaş (2001), a high lignin content leads to a higher CV, it was expected that due to the observed lignin degradation in aerobic pretreated straw–compost mixtures (Beuel, Rieker, and Bursche 2020), the CV should decrease. In fact, the pellets subjected to aerobic incubation (SCW_2) showed the lowest GCV of all examined samples, with an average value of 12.6 MJ kg^{-1}. However, the pellets subjected to aerobic incubation combined with autoclaving at 120 °C and 140 °C had GCVs of 15.9 and 16.7 MJ kg^{-1}, respectively. It seems that lignin degradation may represent a contributing factor to the reduction in calorific value, as evidenced in SCW_2. At this stage, a categorical conclusion cannot be drawn because SCW_5 and SCW_8 were subjected to similar treatments and the ANOVA analysis (Appendix, Table S8) indicates that the incubation variable does not affect the CV. The relative standard deviation between the replicates of samples SCW_2, SCW_5 and SCW_8 range from 1.1 to 3.7%. Hence, it is hypothesized that this difference in GCV for aerobic treatment may have other causes and further studies considering chemical and microbiological composition of samples should be carried out. Lignin is characterized by its high carbon content, so its content probably varies along with the degradation and ultimately affects the GCV (Demirbaş 2001; Yan et al. 2019). However, during thermal pretreatment the temperature has the strongest influence on the increase of CV and may counteracts any lignin related differences (Scherzinger, Kulbeik, and Kaltschmitt 2020).

Singhal et al. (2001a) determined an average improvement of only ≤ 0.5 MJ kg^{-1} in NCVs after washing processes, therefore the effects on CV are rather negligible. Comparing the average GCV of SW pellets (17.6 MJ kg^{-1}) and SCW pellets (15.3 MJ kg^{-1}), the GCV of the latter is about 13% lower. This is to be expected because of the higher ash content of the SCW samples and lower calorific value of the compost. Further studies should be conducted to further quantify the described effects on the calorific value as well as adjust the percentage of compost mixed with straw. Finally, unlike the SCW pellets, the calorific values of the SW pellets comply with the industrial standards according to ISO 17225-6, as their NCV values are always above 14.5 MJ kg^{-1}.

5.4.3 Ash melting behavior

The ash melting range is assumed to extend from DT to FT, with the melting range extending from DT to HT. The range extending from HT to FT is considered to be the flow range (Saddawi et al. 2012). It was decided to focus on SST and DT as they are important indicators of ash deposition behavior (Toscano et al. 2019; Deng, Zhang, and Che 2013). According to the ISO 21404, the SST is the stage at which the first signs of partial melting are observed, causing very serious problems when using this type of fuel in small boilers (Matúš et al. 2018; Deng, Zhang, and Che 2013). According to Toscano et al. (2019), DT should also be in focus, because this temperature coincides with the critical phase in which the ash begins a series of physical changes. The characteristic ash temperatures for washed SW and SCW mixtures as well as untreated straw and compost are summarized in Table 15.

Table 15: Average ash-fusion temperatures of washed samples (°C).

Code	Samples	SST	DT	HT	FT
SW_1	SW30_Control	748	>1,500	>1,500	>1,500
SW_2	SW30_I	724	1,342	1,386	1,422
SW_3	SW30_B	778	>1,500	>1,500	>1,500
SW_4	SW30_a120Control	770	>1,500	>1,500	>1,500
SW_5	SW30_a120I	746	1,268	1,454	1,500
SW_6	SW30_a120B	783	>1,500	>1,500	>1,500
SW_7	SW30_a140Control	768	>1,500	>1,500	>1,500
SW_8	SW30_a140I	719	1,437	1,455	>1,500
SW_9	SW30_a140B	783	>1,500	>1,500	>1,500

Code	Samples	SST	DT	HT	FT
SCW_1	SCW30_Control	1,185	1,455	>1,500	>1,500
SCW_2	SCW30_I	1,168	1,455	>1,500	1,500
SCW_3	SCW30_B	1,215	>1,500	>1,500	>1,500
SCW_4	SCW30_a120Control	1,184	>1,500	>1,500	>1,500
SCW_5	SCW30_a120I	1,192	>1,500	>1,500	>1,500
SCW_6	SCW30_a120B	1,368	>1,500	>1,500	>1,500
SCW_7	SCW30_a140Control	1,163	1,500	1,500	>1,500
SCW_8	SCW30_a140I	1,164	1,411	1,445	>1,500
SCW_9	SCW30_a140B	1,268	>1,500	>1,500	>1,500
S	Straw (untreated)	710	1,153	1,391	1,434
C	Compost (untreated)	1,189	1,441	>1,500	>1,500

Ash from untreated straw had the lowest average values of characteristic temperatures and is within the range of literature (Toscano et al. 2019; Nosek et al. 2020; Matúš et al. 2018). Then there is a gradual increase in the SST of the SW samples, showing an average of 757 ± 24 °C, equivalent to an improvement of 6.6% over straw without pre-treatment. Pure compost has an SST of 1,189 °C. For the samples of a homogeneous mixture of SCW, the SST values remain above 1,163 °C. This leads to significantly higher SST values than for the SW samples, corresponding to an average increase of 60% compared to the pretreated SW samples (Figure 20). In addition, some ash specimens reached DT, HT, and FT above the maximum measurement temperature of > 1,500 °C.

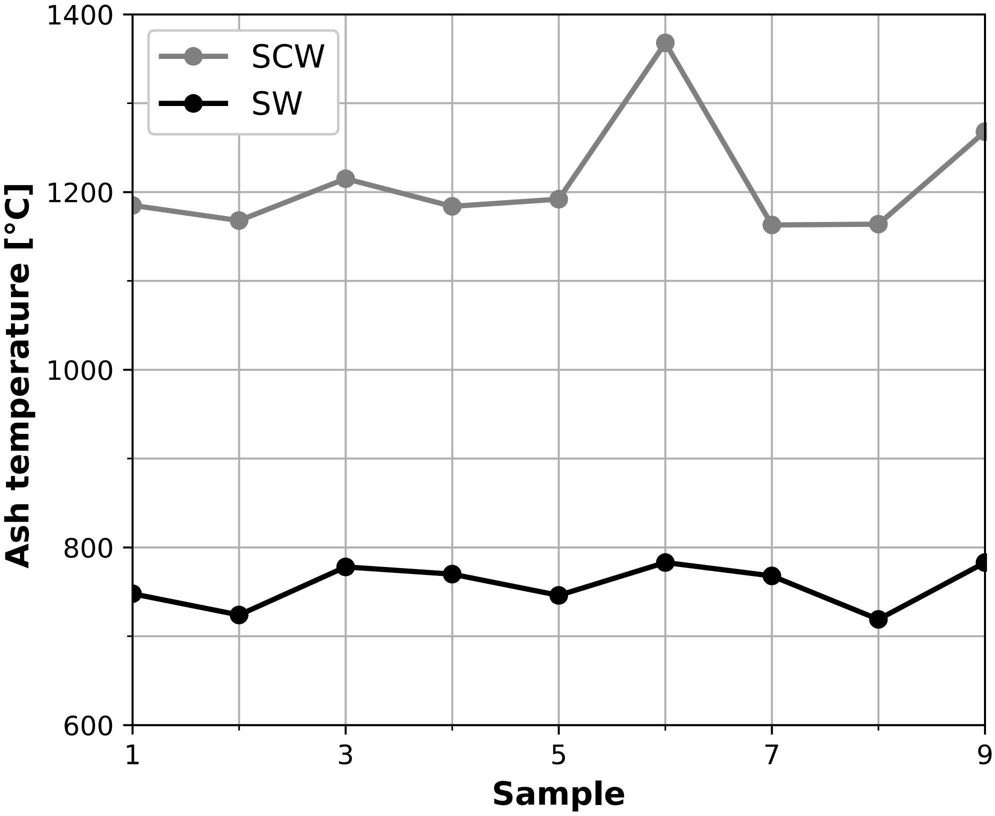

Figure 20: Comparison of SST of pretreated and washed SW and SCW samples.

Only the main effects of incubation were statistically significant for the influence on SST of SW (Appendix, Table S9) and SCW samples (Appendix, Table S10). Regarding to DT, the ANOVA revealed that for the SW samples only incubation (Appendix, Table S11), but for the SCW samples both autoclaving, incubation and interaction between pretreatments showed statistical significance (Appendix, Table S12). Accordingly, when the main effect of incubation on SST or DT is analyzed, the results depend on the type of incubation performed. For example, an average improvement in SST of 4.4% for SW and 8.3% for SCW samples was observed for ashes subjected to anaerobic treatment compared to ashes not subjected to thermal treatment. On the other hand, the same comparison for ashes subjected to aerobic treatment showed an average decrease in SST of 2.5% for SW and 0.9% for SCW samples. For the aerobic SW samples, DT decreased on average by 10.1% compared to SW30_1. The shape changes of ash specimen from anaerobic samples of SW and SCW at SST are shown in Figure 21. The cylindrical ash specimens did not change their shape significantly when the temperature was increased up to the test maximum of 1,500 °C.

Figure 21: Shape changes of the specimen from ash of
(A) SW30_a140B and (B) SCW30_a140B at SST; DT, HT and FT > 1,500 °C.

The results indicate that the anaerobic incubation pretreatment had a positive effect on SW and SCW materials by causing an increase in both SST and DT. The increase in SST and DT after anaerobic incubation at 55 ◦C could be explained due to higher micro-nutrient requirements for thermophilic systems in respect of mesophilic systems, which led to a higher removal of K, Cl, S, and Na (Romero-Güiza et al. 2016). On the other hand, aerobic incubation pretreatment decreased SST and DT. Due to lignin degradation during aerobic incubation and because lignin is known to be more thermally resistant compared to carbohydrates, SST and DT are expected to decrease as a result (Yan et al. 2019). The results are considered satisfactory, since a significant increase in ash temperatures was obtained for both sets of samples (SW and SCW). Consequently, the melting temperatures increase due to the thermo-biological pretreatments applied, as shown in Figure 22, which prevented the characteristic temperatures for determining the ash melting behavior from being observed.

Figure 22: Ash samples after testing the ash melting behavior until the measuring range of 1,500 °C is reached:
(A) Untreated wheat straw (B) SW30_a140B (C) SCW30_a140B.

Moreover, similar results to those presented here were found in the literature for pretreated wheat chaff, i.e., SST around 800 °C and values for DT, HT, and FT above 1,500 °C (Weiß and Glasner 2018). In comparable literature studies, wheat straw was mixed with paper sludge, and results above 1,025 °C and 1,080 °C, respectively, were obtained for DT (Nosek et al. 2020; Matúš et al. 2018). However, the levels found in this study for the SCW samples are still higher and would not cause ash sintering and slagging (Toscano et al. 2019; Nosek et al. 2020; Matúš et al. 2018). In particular, HT and FT values are comparable to the melting temperature of wood and can significantly reduce the technical effort for combustion applications (Weiß and Glasner 2018). According to Vassilev et al. (2013), it can be considered that the SCW samples investigated in this study have moderate (1,200 – 1,400 °C) to high (1,400 – 1,600 °C) ash melting temperatures among biomasses. Nevertheless, due to the low sintering temperature (< 800° C, Table 15) and low ash contents of the SW samples (0.5 – 4.0%, Table 13), it is expected that the use of a moving grate will be required for pellet combustion (Weiß and Glasner 2018).

As already explained in section 5.3.3.5 and 5.4.1, washing removes minerals such as K, Na, Cl or S, which reduce the melting temperatures of straw. As shown in Table 13, the average ash content of the SCW samples decreased from 39.1% to 22.4% after washing, which is a 43% reduction. Consequently, the melting temperatures, especially DT, increased, which can be mainly explained by the low K content due to the washing process (Vassilev et al. 2017; Saddawi et al. 2012; Deng, Zhang, and Che 2013; Weiß and Glasner 2018). The achieved ash melting temperatures are beneficial for combustion, but opposite effects due to the described compost mixture were also observed in the SCW samples, i.e., increased ash content and decreased CV. Therefore, for future research, it is suggested to vary the percentage of compost added to the wheat straw to find the optimal point between these three variables.

5.5 Conclusion

The effects of thermo-biological pretreatment on the combustion properties such as ash content, calorific value, and ash melting behavior of wheat straw were investigated. For the SW pellets, the ISO 17225-6 standard is met after pretreatment. These would be suitable for combustion processes in small boilers that may require additional components. For medium or large boilers, pretreated SW pellets could be used with- out further adjustments. On the other hand, the suitability of compost as an additive was investigated and different reactions were observed. Although the melting temperatures – specially SST – were significantly increased to avoid combustion problems, negative effects occurred on ash content (increased) and GCV (decreased). These negative effects were largely reduced, for example, by autoclave treatment at 140 °C in conjunction with the washing process; however, for SCW pellets the standard ISO 17225-6 is not met. Nevertheless, autoclave and anaerobic incubation combined with the washing process were shown to be a promising pretreatment of wheat straw for pellet production. The key findings of this study can be summarized as follows.

- For both set of samples, a reduction in ash content was observed after pretreatment, up to 37% for the SCW samples after pretreatment in an autoclave at 140 °C. Additionally, a drastic reduction in ash content was observed after the washing process, on average about 55% (SW samples) and 43% (SCW samples).
- An average improvement of 10% in calorific value was observed in SCW samples after autoclave pretreatment at 140 °C combined with incubation regardless of type. In contrast, no statistically significant improvement in calorific value was observed in the SW samples as a result of pretreatment, however, their NCV values were always above 14.5 MJ kg^{-1}.
- Anaerobic incubation pretreatment at 55 °C had a positive effect on SW and SCW samples by increasing the SST. In all cases in this study, all anaerobic pretreated ash specimens reached DT, HT, and FT above the maximum measurement temperature of > 1,500 °C. Autoclave treatment at 140 °C and anaerobic incubation at 55 °C in conjunction with washed straw–compost mixtures is a possible solution to problems associated with low ash melting temperatures, as the shrinkage temperatures were increased over 659 °C compared to raw straw.
- The potential of compost is promising and qualifies this material as a possible additive. For future studies, it is recommended to reduce the compost ratio to diminish the counterproductive effects such as the high ash content and low calorific value. SST can serve as a reference and should remain at a moderate level above 1,000 °C after appropriate ratio adjustment. Higher autoclave temperatures might additionally contribute to raise the GCV of the straw–compost mixtures.
- The cascading use of straw–compost mixtures increases the value-added opportunities of a biorefinery, since solid fuels with optimized combustion properties can be provided in addition to liquid or gaseous fuels.

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6 General results and discussion

In this chapter, the results are merged within the framework of a Life-Cycle-Assessment, reviewed, and discussed based on the defined research question and hypotheses.

6.1 Life-Cycle-Assessment of the developed pretreatment method

The aim of this thesis was the development of a combined pretreatment concept for lignocellulosic biomass to utilize all biomass fractions in order to maximize the product yield per biomass input. By comparing the investigated configurations of the developed pretreatment concept, the overall research question of this thesis will be answered to a large extent. Thus, the LCA can be used to estimate the environmental impact of the production and fractionation of the lignocellulosic feedstock using the different configurations from Chapter 4. The methodology developed in Chapter 3 was applied for the comparative analysis of pretreatment processes for wheat straw lignocellulose according to environmental and technical criteria. The results of the already evaluated and compared (reference) pretreatment processes (Liquid-Hot-Water and acetone-based Organo-Solv) from Chapter 3 were included in the extended techno-ecological evaluation.

6.1.1 Assessment framework and quantification of the processes

Wheat straw is the feedstock for all processes. Since the fractionated lignocellulosic components differ both in quantity and quality, the reference per kilogram dry matter (DM) or the dimension 1 kg DM wheat straw was used as the functional unit for the life cycle inventory. All relevant mass flows, auxiliary materials and energy requirements were classified. The scope of investigation and the resulting system boundary for the investigated processes were determined on the basis of ISO 14040/14044 and VDI 6310 guideline. In order to ensure equal conditions for the investigated pretreatment processes, the elemental and energy flows were equally considered both during the provision of the raw material wheat straw and during its transport. The LCA study was carried out according to the "cradle to gate" approach. Therefore, the analysis starts with the raw material wheat straw and ends, as in Chapter 3, after the separation of the fractions (sugar derivatives of cellulose and hemicellulose, and lignin in liquid and/or solid form). Subsequent process steps, such as biogas or bioethanol production, were not considered in this LCA study, since the focus of this thesis was on process development and its performance with regard to fractionation of lignocellulose. Based on this, the objectives and scope of investigation were defined for the processes to be examined for the supply of the lignocellulosic components.

It is assumed that the wheat straw is stored at the processing site until the required water content (7.2%) is achieved. In order to create the best possible comparability with other processes, the energy flows were calculated using material-specific data such as the specific heat capacity. Transport routes between the individual process steps were not included. For process steps without concrete data about the machines used, reference machines were obtained by means of external data research (e.g., for mixing wheat straw with water, digestate and compost). Environmental impacts entering or leaving the system boundary were taken into account, including the production process of the electric current. The balance sheet framework shown in Figure 23 results from the definition of the scope of the study and the assumptions.

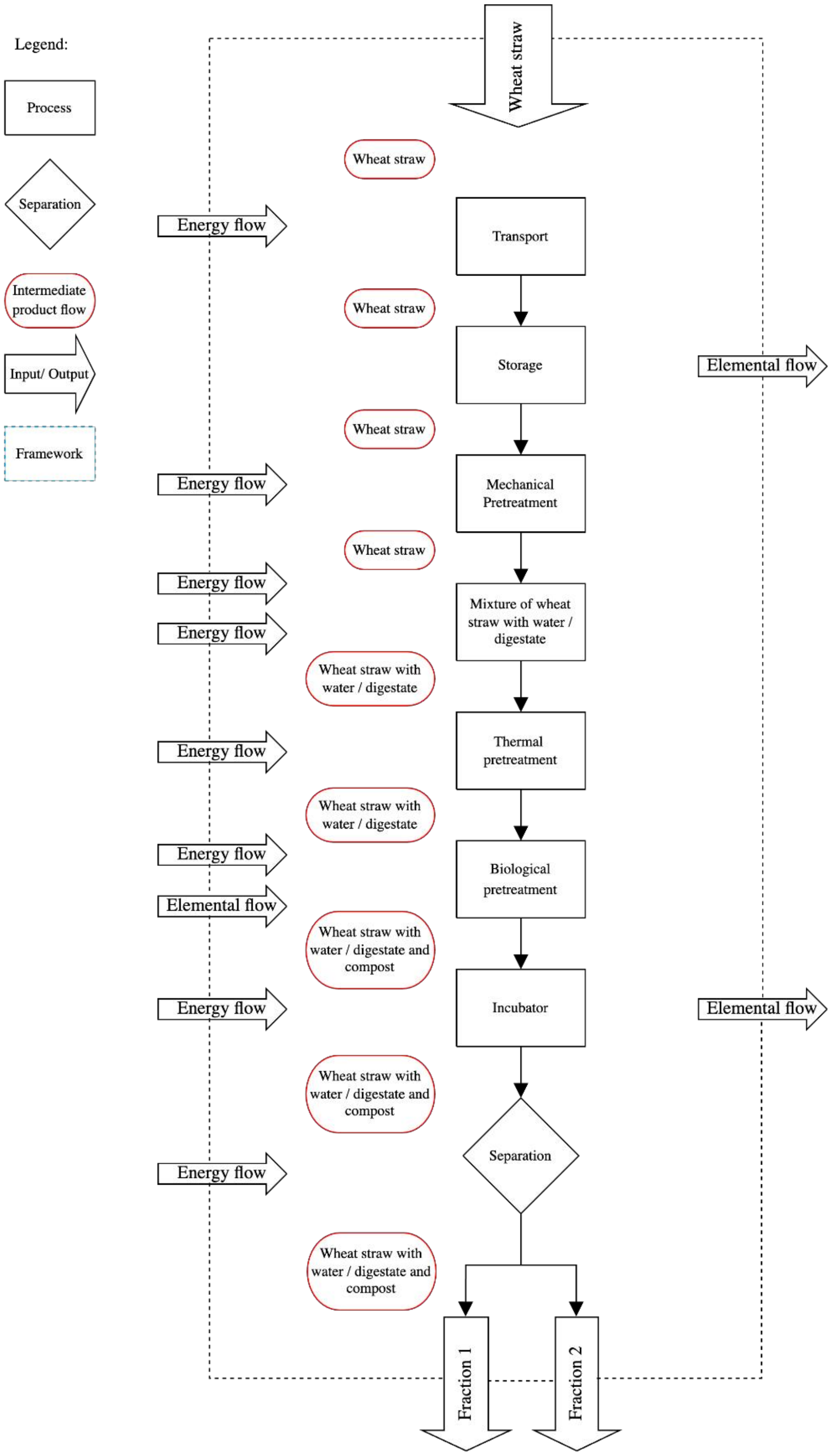

Figure 23: Framework of the thermo-biological pretreatment method from Chapter 4 according to ISO 14040 and VDI 6310.

The energy and elemental flows of the individual process modules determined in the life cycle inventory for the individual pretreatment methods (SCWa-I; SCDa-I; SCWa- B; SCDa-B) and the flow directions (input, output) are listed in Appendix, Table 24.

According to ISO 14040/14044, allocation procedures are required when dealing with systems in which products are reused, such as green waste compost (as a natural source of microorganisms) or the liquid digestate (as a substitute for an acidic solution with a high concentration of ammonium ions). Allocations are based on physical-causal relationships, common physical parameters (mass or heating values), and/or the economic values of the valuable outputs of the multi-output process (Luo et al. 2009; ISO 2006a; 2006b). In this thesis, the allocation is based on mass. To ensure the functional equality of the systems, equivalence processes are determined in a first step (ISO 2006b). The equivalence processes are primary processes that are saved by reuse. Under certain circumstances, this can lead to negative environmental effects being obtained as a result (Geldermann, Schmehl, and Hesse 2012; Croes and Vermeulen 2021; Corona et al. 2018; Luo et al. 2009).

Pretreatment with an ammonia solution is therefore used as an equivalent. Liquid digestate with high $NH4^+$-N concentration (3.75 g $NH4^+$-N kg^{-1} LD^{-1}, Liu et al. 2019) replaces ammonium production and therefore a commercial production process of mineral fertilizers (ammonium nitrate, 33.5% nitrogen content) was included from the *GaBi* database to determine benefits (Geldermann, Schmehl, and Hesse 2012). Based on the ammonium concentration of the liquid digestate in Liu et al. (2019), the ratio was calculated in relation to the amount of liquid digestate used. A prerequisite for the integration of benefit effects into the Life-Cycle-Assessment is that the causal effects and consequential effects can be determined and described not only qualitatively, but also quantitatively (Kehrens 2012). Based on the properties of the used green waste compost observed in Chapters 4 and 5, comparable to the effects of biological pretreatment reported in Potumarthi et al. (2013), where enzymes (such as cellulase, xylanase, lignin peroxidase, glyoxidase etc.) were formed during the fungal treatment, a commercial enzyme manufacturing process was selected as an equivalent process for the allocation process. Due to limitations in the LCA database, enzyme production (glucose-based) was therefore selected. Based on the conversion ratio in Vasco-Correa and Shah (2019) (0.5 kg fungal biomass per kg glucose), the enzyme mass ratio was calculated in relation to the amount of compost used and glucose content of wheat straw.

6.1.2 Selection of impact categories

The life cycle inventory phase is followed by the impact assessment. This was performed by the LCA software *GaBi* using the impact assessment method *ReCiPe* (2016). Six impact categories were selected from the 18 available midpoint categories for the extended LCA study. Global warming potential (GWP) was examined as an important and timely impact category in this LCA study. Another important category is the human toxicity (HTP). The impact categories freshwater eutrophication (FEP), freshwater toxicity

(FETP) and terrestrial acidification (TAP) were additionally selected to represent the effects of the investigated pretreatment process as completely as possible. Due to the increasing depletion and limited availability of fossil raw materials, the impact category fossil degradation (FDP) was also selected. These impact categories have also been used or recommended in comparable LCA studies as well as in studies evaluating biogenic process chains (Emeder 2021; Jeswani, Falano, and Azapagic 2015; Corona et al. 2018; Prasad et al. 2016; Cristóbal et al. 2016; Beylot et al. 2015; Ingrao et al. 2021; Seghetta et al. 2016).

6.1.3 Results and discussion

The results of the Life-Cycle-Assessment are shown in Table 16. After ecological analysis of the developed thermo-biological pretreatment process, it can be stated that the main emitter is process energy in the form of electrical energy in almost all impact categories. The greatest demand is due to the 14-day incubation. For this purpose, anaerobic incubation (at 55 °C) has a higher energy demand by a factor of 10 than aerobic incubation (at 25 °C). In the GWP category, for example, about 95% of the total kg CO_2-equivalent in anaerobic pretreatment can be attributed to electricity. The influence of electricity is also significant in the FETP impact category. For the aerobic pretreatment methods, the use of electricity is responsible for about 65% of the total kg 1,4-DCB equivalents, wheat straw for about 30% and compost for about 3.5%. In contrast, for the anaerobic pretreatment methods, the use of electricity is responsible for about 95% of the kg 1,4-DCB equivalents released. Lower temperatures for pretreatment, e.g., between 25 and 30 °C, are therefore considered as an advantage due to the lower energy requirement (Vasco-Correa and Shah 2019).

Table 16: Environmental impacts in the selected impact categories of the developed pretreatment method according to *ReCiPe* (2016).

Impact category	**Unit**	**SCWa-I**	**SCWa-B**	**SCDa-I**	**SCDa-B**
GWP	kg CO_2-equiv. kg^{-1} DM^{-1}	6,58	30,9	6,91	31,3
FEP	kg P-equiv. kg^{-1} DM^{-1}	4,53E-05	6,00E-05	4,54E-05	6,02E-05
FETP	kg 1.4-DCB-equiv. kg^{-1} DM^{-1}	2,03E-03	8,51E-03	2,12E-03	8,60E-03
HTP	kg 1.4-DCB-equiv. kg^{-1} DM^{-1}	0,149	0,721	0,157	0,729
TAP	kg SO_2-equiv. kg^{-1} DM^{-1}	5,45E-02	6,56E-02	3,15E-02	7,01E-02
FDP	kg oil-equiv. kg^{-1} DM^{-1}	1,77	9,18	1,87	9,29

The use of compost as a recycled product has a positive effect in the impact categories FEP, HTP and FDP. The use of liquid digestate has a greater impact on the impact categories GWP and FEP, due to methane and ammonia emissions along the agricultural life cycle stages of the slurry and the extracted digestate, respectively (Geldermann, Schmehl, and Hesse 2012; Beylot et al. 2015). Other reports have also mentioned the role of nitrogen fertilizers in determining environmental impacts of biomass cultivation (Mandegari, Farzad, and Görgens 2018).

By including the equivalence processes discussed in section 6.1.1, significant environmental credits could be applied to the GWP, HTP, and FDP categories as shown in Table 17.

Table 17: Environmental impacts inclusive environmental savings in the selected impact categories of the developed pretreatment method according to *ReCiPe* (2016).

Impact category	**Unit**	**SCWa-I**	**SCWa-B**	**SCDa-I**	**SCDa-B**
GWP	kg CO_2-equiv. kg^{-1} DM^{-1}	5,39	29,71	4,79	29,18
FEP	kg P-equiv. kg^{-1} DM^{-1}	-5,51E-05	-4,04E-05	-5,53E-05	-4,05E-05
FETP	kg 1.4-DCB-equiv. kg^{-1} DM^{-1}	9,10E-04	7,39E-03	9,77E-04	7,46E-03
HTP	kg 1.4-DCB-equiv. kg^{-1} DM^{-1}	0,06	0,63	0,06	0,63
TAP	kg SO_2-equiv. kg^{-1} DM^{-1}	5,22E-02	6,33E-02	2,82E-02	6,68E-02
FDP	kg oil-equiv. kg^{-1} DM^{-1}	1,36	8,77	1,13	8,55

Negative values represent environmental savings, for example in the FEP category, while positive values show burdens to the environment (Luo et al. 2009; Geldermann, Schmehl, and Hesse 2012; Kehrens 2012). The credits associated with the substituted enzyme dominate the overall evaluation because a source of microorganisms was used in each configuration. Along with the credits associated with ammonia replacement, the lowest impacts were observed for configurations with liquid digestate. Alkali pretreatment, e.g., with ammonia, is popular because of its strong pretreatment effects and relatively simple process scheme, but the main supply chain issue is the high energy consumption and associated greenhouse gas emissions (Ingrao et al. 2021). As a result, the environmental savings in GWP are particularly high in the configuration with LD.

Considering the comparison matrix according to VDI 2225 elaborated in section 3.4.5 and extended for this chapter, the results of the technical-ecological comparison (including environmental savings) are presented in Table 18. The pretreatment methods examined in Chapter 3 were also considered. The ratio of technical and environmental criteria is 45% to 55%. The focus of the technical criteria tends towards fermentable sugar fractions, whereas the focus of the ecological criteria is on climate change (GWP), eutrophication, acidification and fossil degradation due to the biomass cultivation and the associated environmental impacts. The raw data of the conversion rates are discussed and compared in section 6.3. An overall summary of the techno-ecological comparison is provided in the Appendix, Table 25.

Table 18: Techno-ecological comparison of the pretreatment method developed in this work with selected reference methods (including environmental credits).

			Weighting					
			Thermo-biological				**Physical-chemical (Hydrothermal)**	**Chemical (organic solvent)**
			SCWa-I	**SCWa-B***	**SCDa-I**	**SCDa-B***	**LHW**	**Aceto-Solv**
Technical Criteria	**Quantity Glucose**	**10%**	80%	78%	70%	38%	100%	78%
	Availability Glucose	**6%**	50%	50%	50%	50%	75%	100%
	Quantity Xylose	**7%**	78%	75%	100%	44%	27%	27%
	Quantity Arabinose	**7%**	68%	68%	68%	69%	34%	28%
	Availability Xylose, Arabinose	**6%**	40%	60%	40%	60%	100%	80%
	Quantity Lignin	**4%**	72%	82%	58%	100%	36%	29%
	Availability Lignin	**5%**	20%	40%	20%	40%	60%	100%
Ecological Criteria (incl. Environmental credits)	**Global Warming Potential**	**15%**	89%	16%	100%	16%	37%	7%
	Freshwater Eutrophication	**10%**	100%	73%	100%	73%	-89%	-2459%
	Freshwater Toxicity	**5%**	96%	12%	89%	12%	100%	2%
	Human Toxicity	**5%**	98%	9%	100%	9%	22%	1%
	Terrestrial Acidification	**10%**	34%	28%	63%	26%	100%	20%
	Fossil Degradation	**10%**	83%	13%	100%	13%	21%	3%
		Results	**72%**	**45%**	**78%**	**39%**	**44%**	**-214%**

*Partial conversion of sugars to methane during anaerobic incubation.

The techno-ecological comparison showed that the thermo-biological configuration SCDa-I performs best overall with 78% (Table 18). This is due to the high quantity of xylose and moderate glucose yield on the technical side and the low ecological impact in all impact categories except terrestrial acidification. This impact category is largely determined by the use of the compost, but is balanced by the consideration of both equivalent processes and the low energy requirement compared to the other thermo-biological configurations.

In contrast, the chemical pretreatment method Organo-Solv based on acetone, despite good results in the technical criteria, performs worst with -214% (Table 18). This is due to the much higher environmental impacts during the process, with acetone usage providing the most significant impact on the FEP category. The high demand of process energy, the use of acetone and the complex process design for the separation and recycling of fractions, including acetone, are responsible for the high environmental impacts (Smit and Huijgen 2017; Raita et al. 2017).

In second place is the SCWa-I configuration with 72% (Table 18). The technical criteria are comparable to the SCDa-I configuration. However, due to the use of the liquid digestate in SCDa-I and the resulting environmental savings, the performance is more favorable, especially in the GWP, TAP and FDP categories. Based on the presented techno-ecological comparison, the aerobic configurations (SCWa-I and SCDa-I) showed the best overall performance. In contrast to the aerobic configurations, the amount of sugars, mainly glucose and xylose, was lower in the anaerobic configurations, probably due to the observed methane yield during anaerobic incubation described in Chapter 4. However, the amount of lignin in the anaerobic configurations was higher than in the aerobic configurations due to the increased lignin degradation rate discussed in Chapter 4, which is caused by the process conditions emulating the rotting process of green waste compost.

The SCWa-B configuration (Total 45%, Table 18) has good results in the technical criteria, especially quantity and quality of sugars can be highlighted, but scores intermediate in ecological criteria due to the high energy demand during the 14-day pretreatment. In addition, as reported in Chapter 4, some of the sugar fractions have already been converted to methane (160 L kg^{-1} DM^{-1}). A reduction of the pretreatment time while maintaining the same quantity and quality should be targeted in order to achieve a better environmental performance (Sindhu, Binod, and Pandey 2016; Isroi et al. 2011; Mutschlechner, Illmer, and Wagner 2015; Vasco-Correa and Shah 2019).

The studied LHW shows good results in the technical criteria, especially in the amount of glucose, availability of xylose and arabinose, and moderate lignin content (44%, Table 18). In the ecological criteria, the LHW method is behind the thermo-biological configurations, with the exception of SCDa-B. In particular, the impact categories GWP, FEP and FDP are pivotal, which is due to the demand for process energy (electricity for heat and pressure). Compared to chemical applications, such as pretreatment with dilute acid or the acetone-based Organo-Solv process presented in this thesis, the LHW method nevertheless offers significant advantages in terms of Life-Cycle-Assessment and also achieves high sugar availability (Den et al. 2018).

The SCDa-B configuration had the lowest performance at 39%, with performance comparable to the SCWa-B on environmental criteria, but technically in the midrange (Table 18). As reported in Chapter 4, there was a paradigm shift in the degradation of the lignocellulosic material of the samples pretreated with LD because, contrary to expectations, an inhibition process may have occurred instead.

Based on the techno-ecological evaluation of the pretreatment methods investigated, it is evident that biological, chemical and hydrothermal pretreatment processes have different advantages and disadvantages as well as varying suitability for the different areas of application. The developed and analyzed thermo-biological pretreatments are more suitable for energetic applications due to the low technical availabilities (Table 18). For material applications of the dissolved lignocellulosic components, further process steps would have to be implemented, which would probably have a negative impact on the Life-Cycle-Assessment (Tan et al. 2021). Although the aerobic configurations performed better than the anaerobic ones in the LCA, the reduced technical degradation time (SCWa-B; T_{80} of 91%) and the optimized ash melting behavior (SCWa-B; SST > 1,000 °C and DT > 1,500 °C) were demonstrated in Chapters 4 and 5. So, the anaerobic configurations appear more advantageous from a technical point of view – especially with regard to the research question and aim of this thesis, where the development of a thermo-biological pretreatment is intended to increase the conversion rate and efficiency in the utilization of lignocellulosic wheat straw.

However, the techno-ecological comparison also revealed the weaknesses of the developed pretreatment methods with a biological approach, namely the limited flexibility of the possible applicability of the obtained lignocellulosic fractions, the comparatively high energy demand in connection with the long retention times. With the exception of the high energy demand, these results are also largely consistent with recent literature (Akyol et al. 2019; Anukam and Berghel 2021; Galbe and Wallberg 2019; Kumar and Sharma 2017; Tan et al. 2021; Theuretzbacher et al. 2015). The LHW and Organo-Solv, on the other hand, are more flexible in their application, have a significantly shorter process time, and in some cases yield highly pure fractions of the lignocellulosic components (Raita et al. 2017; Sidiras and Salapa 2015; Tan et al. 2021). Hydrothermal pretreatment of lignocellulosic biomass appears to be one of the most promising technologies that can be applied at various scales in biomass processing for fractionation and structural modification (Ruiz et al. 2020). Furthermore, the comparison showed that chemical methods are contrary to global sustainability goals and should not be considered for future biorefinery concepts, despite their good technical properties. Nevertheless, further process improvements can also be expected in the area of chemical pretreatment methods, e.g., it has been reported that the use of alkali-catalysts can lower the process temperatures of an acetone-based Organo-Solv process (Raita et al. 2017). However, the environmental friendliness is still questionable due to the chemicals used.

For the development of integrated sustainable biorefineries in a circular bioeconomy, the approaches developed in this thesis should be coupled with the advantages of the LHW process, for example, by increasing the reaction temperature during thermal pressure hydrolysis (Theuretzbacher et al. 2015; Tan et al. 2021). This could reduce the (biological) retention times and increase sugar yields (Theuretzbacher et al. 2015; Sharma, Xu, and Qin 2019). In addition, other positive effects would also occur with regard to combustion properties as already discussed in Chapter 5 (Scherzinger, Kulbeik, and Kaltschmitt 2020).

LCA is a support tool, but the results of an allocation method are in many cases inadequate and can distort the outcome of an LCA (Luo et al. 2009; Croes and Vermeulen

2021; Beylot et al. 2015). Croes and Vermeulen (2021) indicate that current LCAs with negative environmental impacts are generally based on background data or, like this thesis, use default data from databases. Accordingly, there is always the risk that a scenario does not comply with common rules and that greenwashing is practiced. The authors therefore suggest that negative environmental impacts should not be included in LCAs in most cases. If the environmental savings (leading to negative environmental impacts) are excluded without further adjustments to the techno-ecological comparison (see Appendix, Table 26), the ecological advantages of the developed pretreatment method still appear evident. Thus, the inclusion of environmental savings does not diminish the credibility of the LCA, but rather seems to be a helpful addition, when considering the established criteria and research framework (Croes and Vermeulen 2021). Nevertheless, in its current form, the Life-Cycle-Assessment prepared in this thesis reaches its limitations not only in allocation issues, but also in variable multi-input-multi-output systems, such as the consideration of wheat cultivation, the integration of farm manure or digestate and composts (Luo et al. 2009). Consideration of the optimized combustion characteristics of wheat straw after thermo-biological treatment observed in Chapter 5 (especially after the SCWa-B configuration at 140 °C), as well as the shortened technical digestion time observed in Chapter 4 (SCWa-B and SCDa-B), was not practical with the selected LCA framework in conjunction with the technical criteria. In order to establish comparability with the reference processes, both the LCA framework and the technical criteria were limited to the fractionated lignocellulosic components and designed to the functional unit 1 kg DM wheat straw. This ensured equal conditions for the pretreatment processes investigated. In terms of energy, the advantages relate to the SCWa-B configuration, as it was shown in Chapters 4 and 5 that the utilization efficiency of the biomass feedstock is highest in fermentative and thermo-chemical conversion processes.

6.1.4 Conclusion and outlook

The effects of the developed thermo-biological pretreatment were evaluated with respect to the impact categories of global warming potential, human toxicity, freshwater eutrophication, freshwater toxicity, terrestrial acidification, and fossil degradation. It was determined that the main emitter in almost all impact categories is process energy. In comparison with the selected reference processes (LHW and acetone-based Organo-Solv), the developed configurations performed well in terms of ecological as well as technical criteria, whereas the aerobic configurations (SCWa-I and SCDa-I) showed the best aggregate performance.

Overall, the use of biological pretreatments combined with a preceding thermal pressure hydrolysis (in the low-pressure range) had a major advantage over chemical treatment processes in particular, as the additives used (compost and/or liquid digestate), even without applying the mass-based allocation method for equivalence processes according to ISO 14040/14044, led to ecological savings in the Life-Cycle-Assessment. The advantage of the acetone-based Organo-Solv process is the fractionation of the lignocellulosic components cellulose, hemicellulose, and lignin, after which all fractions are available separately from each other. This results in high availability of the process products with good quality, but with questionable environmental compatibility. The LHW process, on the other hand, achieves a better quantity balance of the process products, with the disadvantage of reduced availability of the cellulose and lignin fractions. The environmental impacts can be summarized as moderately beneficial overall, while process energy is the only factor that leads to higher environmental impacts in the GWP, FEP, and FDP categories.

The evaluation framework established in this thesis proved to be a suitable tool to evaluate different pretreatment methods with regard to defined ecological and technical criteria. However, the study also revealed that certain features of the developed pretreatment method could not be incorporated into the evaluation framework, such as the shorter technical digestion time or the optimized combustion properties of the lignocellulose used.

In order to make the best use of the various technical advantages of the LHW method and to minimize the drawbacks of the anaerobic biological compost treatment as much as possible (retention time and energy demand), process adaptations should be considered, which might lead to an overall improved ecological performance. For future process developments based on the approaches presented here, it is recommended to adjust the mixing ratios of straw to compost and to modify the conditions of thermo-pressure hydrolysis, using a higher temperature.

6.2 Transferability to large-scale applications

A future increase of the share of renewable raw materials in the energy industry and in parts of the chemical industry requires the development of advantageous pretreatment processes and subsequent economic analysis (Shahid et al. 2021). In this regard, various factors determine the minimum scale for the feasibility of biorefineries. According to Moncada et al. (2016), the number and quantity of high value-added products, such as antioxidants and flavors, is generally associated with low scale, and in the case of bioenergy (biofuels, electricity, and heat), large-scale production is offset by low value-added. Several indicators are used in the literature to evaluate the economic performance of a biorefinery. Production cost, gross operating margin, and net present value (NPV) are the most commonly used indicators for economic analysis of a biorefinery, but minimum selling price (MSP), return on investment (ROI), weighted average cost of capital (WACC), and modified internal rate of return (MIRR) can also be evaluated (Solarte-Toro et al. 2021).

So-called techno-economic assessments (TEA) aim to evaluate the technical and economic aspects of manufacturing a product. TEA involves quantifying capital and operating costs, taking into account the different technologies involved in the biorefinery (Solarte-Toro et al. 2021). Based on results of laboratory tests for different steps of biomass pretreatment and conversion, a data basis for the techno-economic evaluation for a potential large-scale realization of the process chain is developed (Haase 2012). The use of *Aspen Plus* and other *flowsheeting* software for specific unit operations and calculations allows accurate prediction of the behavior of processes at large scale (Moncada B., Aristizábal M., and Cardona A. 2016). In this context, pretreatment is considered one of the main factors influencing the cost of the final biorefinery products and therefore requires a certain level of technical maturity already at a laboratory scale (Yang and Wyman 2008).

TEA has already been used to study different types of biorefineries, and this thesis can only present a small selection of studies due to the large number involved, e.g., for salix and spruce (Sassner, Galbe, and Zacchi 2008), corn stover (da Silva, Torres Ortega, and Rong 2016; Sassner, Galbe, and Zacchi 2008; Vasco-Correa and Shah 2019; Humbird et al. 2011), sugarcane bagasse (Mandegari, Farzad, and Görgens 2018; Vasconcelos et al. 2020), and wheat straw (Voutilainen, Pihlajaniemi, and Parviainen 2021; Shafiei et al. 2013; Vasco-Correa and Shah 2019). The prices assumed for the feedstocks in the listed studies vary between 0 and 196 € t^{-1} DM^{-1}. The price for wheat straw was in the range of 40 – 98 € t^{-1} DM^{-1} and thus in the lower range of the prices cited in section 2.1.2 (0 – 150 € t^{-1} DM^{-1}). The economic behavior of a biorefinery is significantly influenced by price fluctuations of raw materials (Solarte-Toro et al. 2021). Storage and transportation are two other key factors, because dry biomass has a low bulk density, while fresh biomass has a water content of up to 90%, making it very expensive to transport in its raw state compared to natural gas and oil (Shahid et al. 2021). In the above-mentioned studies, the existing raw material potential of the respective region was generally taken into account and an operating time of 6,000 – 8,000 hours per year was assumed. Different pretreatment options were simulated, e.g., steam explosion and LHW, with or without additive, AFEX, diluted acid pretreatment, Organo-Solv, and so on. Only the study by Vasco-Correa and Shah (2019) simulated and evaluated a biological (fungal) pretreatment process in terms of techno-economic parameters on different biomasses, including wheat straw. The economic indicator was the production costs of fermentable sugars from lignocellulosic feedstocks. Interestingly, the simulated process setup is similar to the concepts presented in this thesis. After hammer milling, a sterilization step (121 °C for 20 minutes) was performed followed by incubation with white-rot fungi for 28 days under aerobic conditions at 28 °C using cooling water, due to the heat produced by the metabolism of the white-rot fungi. Fixed-bed bioreactors were selected for incubation, and various cellulase cocktails were used for subsequent enzymatic saccharification of the fungal pretreated raw materials. The main difficulties of the fungal pretreatment process were the long pretreatment time, low sugar yield, low bulk density of the feedstock, and sterilization requirements. These factors resulted in high space requirements due to the size and number of units for key processes such as fungal pretreatment, enzymatic hydrolysis, and autoclaving, which had a direct impact on capital costs. Compared with the other studies mentioned, biological pretreatment requires a higher capital investment than dilute acid, steam explosion, LHW, and AFEX pretreatment. The results of the study by Vasco-Correa and Shah (2019) demonstrate that the economic feasibility of biological pretreatment concepts at a biorefinery scale does not appear to be realizable at the current state of the art on a "greenfield" basis and that significant process improvements are still required to achieve attractive product cost targets.

From both an economic and an ecological point of view, advantages can be gained by establishing a biorefinery concept at an existing industrial site in a bottom-up approach. For example, in addition to the general economies of scope which may be associated with integration into an existing industrial site, a landfill opens up the possibility of shifting the infrastructure from a disposal-only paradigm to product manufacturing (Madadian et al. 2021). With the possibility of in-situ material conversion and utilization, for example through composting and biogas production, the necessary plant infrastructure for substrate pretreatment is partially in place. Considering the thermo-biological method of this thesis, the pretreated material can be beneficially converted into biogas with an appropriate biogas plant and the solids can be processed into standard fuels. Such approaches can also be found in the literature: The review by Bolan et al. (2013) reports that landfills can play a role as potential biorefinery sites for biomass utilization and the use of methane as a fuel source. In a recent study, the main objective was to illustrate how locally available municipal waste in Atlantic Canada can be efficiently used as a feedstock for renewable energy production by utilizing the existing infrastructure of municipal solid waste landfills (Madadian et al. 2021). Another study indicated that all biomass accumulating at a landfill site must be used as holistically as possible, ideally as part of a cascading approach to achieve the European zero-waste initiative (Ubando, Felix, and Chen 2020). Therefore, to mimic the biorefinery concept, landfill utilization must be developed, optimized, and intensified (Madadian et al. 2021). As described in section 2.1.2, the regional availability of straw varies significantly and, in contrast to Denmark, there is no established straw market in Germany. Therefore, logistical aspects are essential for the implementation of the concept studied in this thesis. Especially in the vicinity of the *:metabolon* research site, located on a former landfill in North Rhine-Westphalia (NRW), there is a high availability of straw due to extensive agricultural land within a radius of 150 kilometers. Based on the potential study by Zeller et al. (2012), described in section 2.1.2, the straw potential for NRW is between 1.1 and 1.4 million tons of fresh matter (FM) per year. Considering the regional straw supply and compared to abovementioned studies, a wheat straw material input of 40 t DM/h (345,000 t FM or 320,000 t DM per year) could be assumed. Based on the results of this thesis, a process- and cascade-optimized thermo-biological pretreatment process could favor the mentioned shift of landfills and at the same time provide an economic feasibility of a lignocellulosic biorefinery. In the anaerobic configuration, the energy requirement could also be reduced by incorporating waste heat, e.g., from a landfill biogas plant, which might provide economic and ecological benefits (Moncada B., Aristizábal M., and Cardona A. 2016). Nevertheless, under the current conditions, even such a project seems to be associated with high investment costs, but compared to a top-down "greenfield" approach, the conversion and retrofitting of existing biomass-conversion-plants represents a more promising scenario. In addition, the disadvantage of rather energetically oriented biorefinery concepts could be compensated. According to Moncada B. et al. (2016), bioenergy/biofuel production systems struggle to be economically competitive even at large scales, although energetically oriented biorefineries are important because of the high demand for energy products.

The review of Shahid et al. (2021) reported that the European Union and the bio-based industry have invested 3.8 billion euros in biorefinery research and development from 2014 to 2020. However, according to the authors, there is currently no highly effective method of separating the cellulose, hemicellulose, and lignin components of biomass that is commercializable from both an environmental and economic perspective. To compete with petroleum refining, which has dominated for the past 100 years, biorefinery technologies must be further researched and developed to create environmentally friendly and efficient processes.

6.3 Discussion of research question and hypotheses

To conclude the research question, this section discusses the hypotheses raised. An overview of the verified, partially verified, and falsified hypotheses is presented in Table 19. In the following, the results are summarized and discussed based on the established hypotheses and the relevant literature.

Table 19: Overview of tested hypotheses.

Hypothesis	Assumption	Verification
1	The developed pretreatment is a resource-efficient, environmentally friendly and low-energy process compared to established methods.	partially verified
2	The availability and quality of the lignocellulosic components after pretreatment is comparable to established processes.	partially verified
3	Autoclaving causes thermohydrolytic decomposition and affects bioconversion efficiency and combustion properties.	verified
4	Green waste compost as pretreatment additive causes lignocellulose degradation and increases bioconversion efficiency.	verified
5	Liquid digestate as pretreatment additive causes lignocellulose degradation and increases bioconversion efficiency.	falsified
6	There is an improvement of combustion properties due to the biological pretreatments, in addition to the increase of methane yield.	verified

Hypothesis 1: Compared to chemical or physical-chemical pretreatment methods, the developed (thermo-)biological pretreatment is a resource-efficient, environmentally safe and low-energy process.

Ecological comparison of the developed thermo-biological configurations with the selected reference processes, acetone-based Organo-Solv (chemical) and LHW (physical-chemical), partially verified the first hypothesis.

The ecological comparison has demonstrated that both the anaerobic and aerobic configurations can be considered resource-efficient. As a result of the usage of compost (as a natural source of lignocellulose-degrading microorganisms instead of commercial enzymes) and the liquid digestate (instead of an alkaline or acidic solution with lignocellulose-degrading properties), environmental savings could be claimed in the LCA. According to the definition of Fehrenbach et al. (2017), the presented pretreatment concepts represent a cascade use of biomass, as the used bio-based end products were utilized a second time for material and energy application. Consequently, the aerobic configurations are resource-efficient in a multi-stage manner because of the coupled use of liquid digestate and compost.

Apart from the comparatively high energy requirements of the anaerobic configuration, which are negatively reflected primarily in the GWP, HT and FDP impact categories, the developed configurations perform better overall than the comparison processes. This confirms the estimations of the current literature regarding the environmental friendliness of biological pretreatments (Galbe and Wallberg 2019; A. K. Kumar and Sharma 2017; Palvasha et al. 2021; Sindhu, Binod, and Pandey 2016; Tan et al. 2021; Tian, Zhao, and Chen 2018).

The aerobic configurations are characterized by the low energy demand, which further supported the assumptions of recent literature on biological pretreatments (Mutschlechner, Illmer, and Wagner 2015; Sharma, Xu, and Qin 2019; Vasco-Correa and Shah 2019; Sindhu, Binod, and Pandey 2016; Tan et al. 2021; Y. Ma, Shen, and Liu 2020). Due to the 14-day incubation at 55 °C and the resulting energy demand, these assumptions do not match the anaerobic configurations. Shortening the incubation time may be possible by adjusting the temperature of the preceding thermal pressure hydrolysis, which could reduce the overall energy demand and increase the sugar yield (Theuretzbacher et al. 2015; Tan et al. 2021; Sharma, Xu, and Qin 2019). Because of the mentioned benefits of the anaerobic configurations, it would also be conceivable to lower the incubation temperature of the anaerobic configuration to 25 – 30 ° and increase the process temperature of the preceding thermal pressure hydrolysis, while maintaining the incubation time. If applicable, the advantages of the respective configurations can be combined from a technical point of view – and the ecological impact may still be classified as low. However, Galbe and Wallberg (2019) pointed out that only sterilized material showed signs of degradation (during biological treatment) and thus eliminating the pre-process as in Vasco-Correa and Shah (2019) would not provide any technical benefits.

In summary, it can be stated that, as described and recommended in the literature, combined pretreatments, such as the approaches presented in this thesis, are promising (Corona et al. 2018; Tan et al. 2021; Zhang et al. 2022; Ruiz et al. 2020). For the future, process- and cascade-optimized thermo-biological pretreatments appear to be the most suitable to meet the challenges of a circular bioeconomy, as they are the least harmful as well as the most sustainable in terms of ecology.

Hypothesis 2: The availability and quality of the lignocellulosic components cellulose, hemicellulose and lignin after (thermo-)biological pretreatment with green waste compost are comparable with established chemical or physical-chemical pretreatment methods.

Technical comparison of the developed thermo-biological configurations with the selected reference processes, acetone-based Organo-Solv (chemical) and LHW (physical-chemical), partially verified the second hypothesis.

When considering the sugar fractions obtained from the lignocellulose after pretreatment in Table 20, apart from xylose and lignin, a comparison with the reference methods shows that the quantities are quite comparable.

Table 20: Comparison of the quantity balances for the lignocellulosic fractions of the investigated pretreatment methods.

Mass balance of pretreatment	**Thermo-biological**				**Physical-chemical (Hydro-thermal)**	**Chemical (organic solvent)**	**Unit**
	SCWa-I[1]	**SCWa-B[1,2]**	**SCDa-I[1]**	**SCDa-B[2]**	**LHW**	**Aceto-Solv**	
Glucose	334	325	292	159	417	325	g kg^{-1} DM^{-1}
Xylose	172	164	220	97	59	60	g kg^{-1} DM^{-1}
Arabinose	34	33	50	35	17	14	g kg^{-1} DM^{-1}
Lignin	400	452	321	554	199	161	g kg^{-1} DM^{-1}

[1] Summarized and averaged values of the results from Chapters 4 and 5
[2] Partial conversion of sugars to methane during anaerobic incubation.

The high lignin content of the straw–compost mixtures is due to the added lignin content of the compost (653.42 – 691.00 g kg^{-1} DM^{-1}). The differences in the amounts of hemicellulose fractions can be attributed to the chosen settings of the reference processes. For example, in the study of Pérez et al. (2007), in which different process variables of the LHW process were investigated to determine the effectiveness of the pretreatment, the composition of the solid and liquid fractions obtained after filtration of the pretreated material was evaluated. In the process run described in Chapter 3 (200 °C, 40 min, substrates loading 1:10 (w/v)), 4.6% of the hemicellulose fraction were recovered in untreated raw material, while other runs recovered up to 52.7%.

With regard to availability, however, the technical comparison disclosed that the reference processes have significantly higher availability and are therefore more flexible in terms of their possible applications (material and/or energy applications). As already described in sections 2.3.3 and 2.3.5, the advantages of chemical processes, especially an Organo-Solv process, are the high efficiency of cellulose and hemicellulose fractionation and the high lignin dissolution. Based on this technology, the EU-funded project *BIOCORE* (BIOCOmmodity REfinery), for instance, was founded in 2010 to demonstrate the industrial feasibility of a biorefinery using a patented Organo-Solv process (Piotrowski et al. 2014). In addition, the technical comparison confirmed that LHW processes are mainly characterized by the high degree of hemicellulose degradation and the good hydrolyzability of cellulose. However, according to Ruiz et al. (2020), further research is needed, as lignin should also be used in LHW-based biorefineries – respectively, there must be the flexibility to prioritize lignin utilization over glucose and xylose.

In contrast to the reference processes, biological pretreatments may result in a loss of sugar fractions because the microbes use nutrients from the same lignocellulosic biomass for their growth and metabolism (Abraham et al. 2020; Y. Ma, Shen, and Liu 2020; Isroi et al. 2011). In the study by Zhang et al. (2022), the effects of ammonia fiber explosion (AFEX) in combination with NaOH (A-NaOH) on the properties of various plant and woody lignocellulosic biomasses were investigated using enzymatic effect analysis. Following the equations used in the study (see Appendix, Figure 26) to calculate the solids and sugar recovery rates for lignocellulosic fractions (taking into account the sugar yields determined by HPLC in Chapter 4 after thermo-biological pretreatment) provides Table 21.

Table 21: Calculated recovery rates of solids, cellulose, and hemicellulose after thermo-biological pretreatment (Chapter 4) adapted from Zhang et al. (2022).

Samples	Solid Recovery (%)	Cellulose Recovery (%)	Hemicellulose Recovery (%)
SCWa-I	99.33 ± 0.23	66.03 ± 1.25	73.35 ± 0.58
SCDa-I	98.49 ± 1.11	63.39 ± 3.94	74.48 ± 2.20
SCWa-B	93.26 ± 2.71	41.73 ± 1.38	45.03 ± 0.88
SCDa-B	92.47 ± 4.48	32.38 ± 2.58	34.91 ± 1.94

It was mentioned earlier that methane was produced in the anaerobic configurations during the 14-day incubation, and thus metabolism occurred during lignocellulose degradation. When considering Table 21, this circumstance appears evident, as the recovery rates of the polysaccharides cellulose and hemicellulose as well as the solid recovery were lower compared to the aerobic configurations.

Furthermore, it can be seen that the metabolic rates in the SCDa-B configuration were higher than in the SCWa-B configuration due to lower polysaccharide recovery rates (Table 21). Nevertheless, the methane yield was lower during the biological pretreatment. This is confirming the assumption made in Chapter 4, that the microbial processes already led to inhibition during the pretreatment and thus no further improvement of biomethanization was observed in the following.

The yield and availability of the obtained lignocellulosic fractions are crucial to design a commercial biorefinery that has both good ecology and economic feasibility. Therefore, further efforts should be made to also demonstrate the technical applicability of the cascaded thermo-biological approach proposed in this thesis in a commercial scale plant. Hence, a key aspect for future research is the one proposed by Y. Ma, Shen, and Liu (2020): the further development of "home-made" highly active enzymes using lignocellulosic waste biomass as feedstock, such as the approach presented in this thesis, to replace commercial enzymes in the pretreatment of straw.

Hypothesis 3: The preceding autoclaving causes thermo-hydrolytic decomposition, which favors a release of the contained sugars and at the same time influences the combustion properties by disintegration of the biomass matrix.

The obtained results in Chapters 4 and 5 verified the third hypothesis.

As shown in Chapter 4, sample SWa (only autoclave treatment at 120 °C) had the highest methane yield (320 L kg^{-1} VS^{-1}). Compared to the raw straw and SW sample, more cellulose and hemicellulose were recovered on average after thermal pressure hydrolysis, resulting in increased methane yield (Table 22).

Table 22: Comparison of the average quantity balances for the lignocellulosic fractions before and after thermal pressure hydrolysis.

Samples	Cellulose g kg^{-1} DM^{-1}	Hemicellulose g kg^{-1} DM^{-1}
Raw straw	408.30	314.22
SW	381.81	314.13
SWa	396.56	320.79

The decomposition of feedstock components such as cellulose and hemicellulose during pretreatment resulted in an increase in hemicellulose content. The increase in hemicellulose content of SWa compared to the untreated substrate (314.22 g kg^{-1} DM^{-1}, Table 22) is an important advantage of biomass pretreatment for subsequent bioconversion, as the material fed to the subsequent hydrolysis step is enriched compared to the untreated material. In studies on the hydrothermal LHW process, accumulations of hemicellulose and/or cellulose were also observed after pretreatment (J. A. Pérez et al. 2008; José A. Pérez et al. 2007).

Furthermore, consistent with the results of Banoth et al. (2017) and the results from Chapter 5 (see Table 23, compare autoclave treatment at 120 °C with 140 °C), more sugar fractions are hydrolyzed at higher temperature than at lower temperature pretreatment.

Table 23: Chemical composition of fractionated samples after thermo-biological pretreatment (Chapter 5).

Samples	**Glucose g kg^{-1} DM^{-1}**	**Xylose g kg^{-1} DM^{-1}**
SCWa30_120_I	330.72 ± 16.21	146.24 ± 5.03
SCWa30_140_I	369.13 ± 30.42	159.50 ± 14.94
SCWa30_120_B	369.65 ± 30.73	172.09 ± 21.18
SCWa30_140_B	403.91 ± 15.42	189.07 ± 10.25

Also, Scherzinger et al. (2020) observed an increase in total biogas production after autoclave treatment of green wastes with a pretreatment temperature of 130 °C. The authors explained this with the occurrence of more complete hydrolysis reactions, i.e., more organic compounds were hydrolyzed from the biomass. Similar to Chapter 5, this study also found an improvement in combustion characteristics with increasing temperature, including a higher calorific value, which was explained by a temperature-dependent decrease in oxygen and hydrogen content and bond dissolution in the biomass matrix, resulting in the removal of compounds with relatively low calorific value, such as hemicellulose. Processes comparable to the autoclave, such as hydrothermal carbonization (HTC), can also positively influence the properties relevant for fuel combustion. In Hansen et al. (2022), after HTC treatment of wheat straw at a temperature of 180 °C, a CV of 18.5 MJ kg^{-1} was observed. In comparison, samples SWa120 and SWa140 (Chapter 5) achieved a CV of 15.9 and 15.7 MJ kg^{-1}, respectively. For wheat straw, the effect of HTC seems to be very beneficial as it also affects the ash melting behavior (especially SST, HTC at 180 °C increases SST by 93 °C compared to raw straw) by a drastic decline of alkali metals after HTC treatment (Cheng et al. 2022; Hansen, Fendt, and Spliethoff

2022). Compared to raw straw in Chapter 5, an increase of SST up to 60 °C was also observed in samples SWa120 and SWa140 (Chapter 5).

In summary, hydrothermal processes are well suited to improve both bioconversion in fermentative processes and the fuel properties of waste streams from lignocellulosic feedstocks such as wheat straw. In comparison with the literature, the results have shown that temperature has the greatest influence. For large-scale applications of the pretreatment shown in this thesis, HTC seems to be advantageous from a technical point of view.

Hypothesis 4: The use of green waste compost as a pretreatment additive causes lignocellulose degradation and increases bioconversion (amount and shortened time) during anaerobic digestion processes.

An improvement in the biodegradability of the lignocellulosic wheat straw by adding compost as a biological pretreatment was observed, resulting in increased methane production with reduced retention time. Consequently, the 4th hypothesis was verified.

The sugar compounds released during biogenic catalysis improved the hydrolysis process – which is considered a rate-limiting step in the anaerobic digestion of solids (Ferreira et al. 2014). During the development of the approach presented here, it was found that a DM content of 30% increased methane yield compared to higher DM contents (Beuel et al. 2021; Bursche, Rieker, and Beuel 2019). Methane production ranged from 270 to 320 L kg^{-1} VS^{-1}, reaching the maximum value after thermal treatment at 120 °C. The combined aerobic configurations achieved methane yields between 270 to 280 L kg^{-1} VS^{-1}, and the combined anaerobic configurations (considering methane yield during pretreatment) 277 to 280 L kg^{-1} VS^{-1}.

The study by Theuretzbacher et al. (2015) included an investigation of the effects of a combination of biological pretreatment and steam explosion on methane yield. The authors used *S. stipitis CBS 5774* for biological pretreatment and recorded a methane yield of 243 L kg^{-1} VS^{-1}. The highest methane yield (254 L kg^{-1} VS^{-1}) was obtained by steam explosion treatment of wheat straw at 180 °C without biological pretreatment. Interestingly, there were no significant differences between the methane yields of samples subjected to combined pretreatment at 180 °C (250 L kg^{-1} VS^{-1}) or 200 °C (252 L kg^{-1} VS^{-1}). Other studies achieved methane yields above 350 L kg^{-1} VS^{-1} with wheat straw, also using the steam explosion at 180 °C (Sołowski, Konkol, and Cenian 2020; Ferreira et al. 2013). However, it should be noted that due to different experimental setups, e.g., different fermentation temperatures, inoculum sources, and dry matter contents in the digesters, the results found in the literature for biological methane potential may vary (Romero-Güiza et al. 2016).

Based on the enhanced bioconversion during anaerobic digestion processes, it can be concluded that the containing microorganisms in green waste compost cause lignocellulosic degradation. For example, the white-rot fungus *P. chrysosporium* can be used in combination with other microorganisms as a compost inoculant for composting lignocellulosic waste. As shown in Figure 24, white-rot fungi have the unique ability to depolymerize, mineralize lignin by ligninolytic enzymes, and cleave carbon-carbon bonds, thereby reducing recalcitation of lignocelluloses and promoting enzymatic hydrolysis (Isroi et al. 2011).

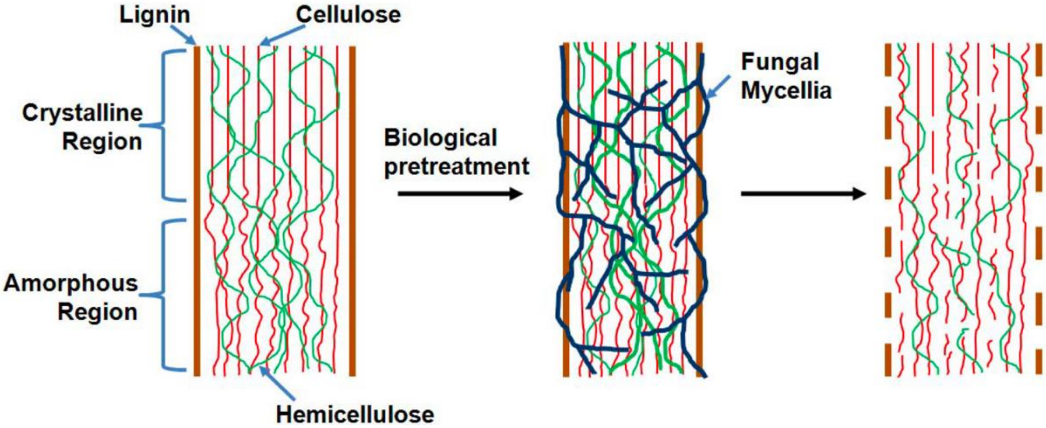

Figure 24: Schematic diagram of biological pretreatment of lignocelluloses. White-rot fungi decrease lignin content and alter chemical and physical structures of lignocelluloses that make biodegradation of lignocelluloses more efficient (Isroi et al. 2011).

Therefore, the study of Li et al. (2020) also investigated whether corn stover can be pretreated with mixed microbes from compost to make biodegradation more efficient. Degradation rates of hemicellulose, cellulose, and lignin were observed to be 20%, 15%, and 13%, respectively, upon compost pretreatment. It can be assumed that the reported degradation rates in the literature may vary due to the different compost types or compositions. The degradation rates of the aerobic configurations reported in Chapter 4 ranged from 3 – 7%, 13 – 21%, and 30 – 36% for hemicellulose, cellulose, and lignin, respectively. For the anaerobic configurations (Chapter 4) for hemicellulose, cellulose, and lignin the degradation rates ranged from 17 – 38%, 25 – 44%, and 0%, respectively. The reasons for the differences between the aerobic and anerobic configurations have already been discussed in Chapter 4. The comparison with the study of Li et al. (2020) reveals the influences of the compost, for example in terms of the different degradation rates of the lignin. The results of the Hemati et al. (2022) study showed that composting material with the highest lignin content resulted in higher respiration activity, spectral absorption, total acidity, C/N ratio, nitrate concentration, pH, and temperature than composting material with lower lignin content, which in turn affected microbial activities and thus biodegradability of lignocellulose. Accordingly, it can be assumed that the variations in the feedstock of composting lead to fluctuating results in lignocellulose degradation.

The T_{80} indicator demonstrated that the fermentation time of the samples pretreated with compost was reduced by 35% to 91% compared to the untreated wheat straw fermentation (Chapter 4). More than 84% of the methane production from wheat straw were already formed during the anaerobic thermophilic biological pretreatment – an advantage over the aerobic configurations, since the mentioned sugar losses due to the metabolic processes (usually conversion to CO_2 and CH_4) are minimized, as the metabolic product methane is captured due to the anaerobic process setting.

In general, it can be stated that adding compost causes lignocellulose degradation and increases bioconversion. Future research should therefore screen different composts for effective microbial strains in different seasons to achieve the maximum degradation rates. This will also build the necessary know-how to contribute to the fulfillment of the research approach mentioned in hypothesis 2 ("home-made" highly active enzymes).

Hypothesis 5: The use of liquid digestate as a pretreatment additive causes lignocellulose degradation and increases bioconversion (amount and shortened time) during anaerobic digestion processes.

Although degradation of structural carbohydrates occurred during incubation with liquid digestate (LD), no further improvement in biomethanization was observed.

Considering recent studies where higher biomethane yields from wheat husk, wheat straw, corn stover, or sugarcane bagasse were obtained by LD coupled pretreatment, it was expected that similar results would be obtained in the experiments presented in Chapter 4 (Sun et al. 2019; Hu et al. 2015; S. Ma et al. 2021; Liu et al. 2019). As discussed earlier in Chapter 4, pretreatment time has the greatest impact on bioconversion efficiency. For example, in Hu et al. (2015), 66.3% more biomethane yield and 41.7% shorter technical digestion time were observed after 3 days of pretreatment compared to untreated straw. According to Sun et al. (2019) and Hu et al. (2015), the optimal pretreatment time is between 3 and 5 days.

It is noted in Liu et al. (2019) that the effects of the equivalent $NH4^+$-N concentration of the ammonia solution pretreatment indicates that ammonolysis plays an important role in the synergy of various substances by LD. Therefore, in the case of digestate, monitoring of the $NH4^+$-N content is necessary to recommend LD for pretreatment of lignocellulosic material.

The LD used in this thesis originates from the post-digester of a pilot plant run with maize silage and cattle slurry at the *:metabolon* research site in Oberbergischer Kreis. The LD from the pilot plant was selected because internal sources at the research facility indicated that it was low-activity material, however, no determination of $NH4^+$-N was performed.

More recent approaches, as presented in Agarwal et al. (2022), instead of incorporating LD into coupled pretreatments (e.g., as a substitute for ammonia pretreatment, with liquid or aqueous ammonia), propose integrated use of digestate through valorization using various thermo-chemical technologies for maximum energy and product utilization within the circular economy model.

With regard to the thermo-biological approaches for wheat straw pretreatment shown in the thesis, it can finally be stated that the use of LD was advantageous from an ecological point of view. However, due to the chosen setup for biological pretreatment with compost (Chapter 4, mesophile aerobic or thermophile anaerobic and 14 days of incubation), no further increase in bioconversion was observed during anaerobic digestion. Accordingly, there are doubts about a positive effect if the substrate is inoculated with LD for more than 14 days. Nevertheless, a high degradation of lignocellulose was observed. As described initially in consideration of relevant literature, the incubation time should be shortened in order to expect an increased bioconversion. However, this could have a negative effect on the degradation rate of lignocellulose and consequently reduce it.

Hypothesis 6: There is an improvement of combustion properties due to the biological pretreatments, in addition to the increase of methane yield.

The results from Chapters 4 and 5 indicated that the use of compost as a biological pretreatment led to improved utilization of lignocellulosic biomasses such as wheat straw. Thus, the sixth and last hypothesis was verified.

Furthermore, it was demonstrated that the cascaded use of straw–compost mixtures increases the value-added opportunities of a lignocellulosic biorefinery concept (tertiary refining), i.e., better utilization of feedstocks with simultaneous production of gaseous energy sources and solid fuels with improved combustion properties.

As already described in Chapter 5, ANOVA revealed that for the SCW samples autoclaving, incubation, and pretreatment interaction showed statistical significance in terms of ash melting behavior and had an effect on calorific value. In terms of calorific value, the use of an autoclave at 140 °C led to better results than autoclaving at 120 °C – up to 10% improvement. Although no statistical significance was determined for the incubation of the SCW samples, in comparison with the SW samples it can be observed that the effect of biological pretreatment with compost in combination with thermal treatment led to a greater reduction in ash content. The experimental washing process, in addition to the pretreatments performed, had a great influence on the ash content and ash melting behavior. Highlighted is the effect on DT as shown in Figure 25, which can be explained mainly by the low K content due to the washing process (Vassilev et al. 2013; Vassilev, Baxter, and Vassileva 2014; Vassilev et al. 2017).

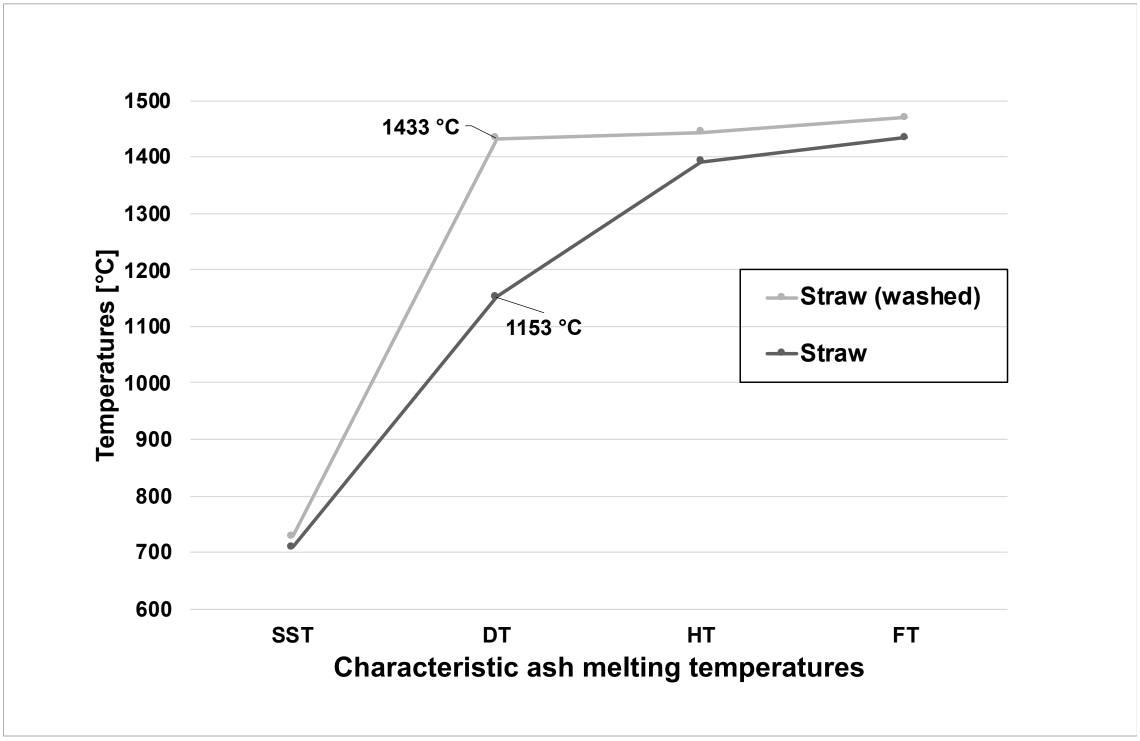

Figure 25: Comparison of ash melting temperatures of washed and unwashed wheat straw.

In a similar study by Hensgen et al. (2011), green waste was sorted into herbaceous, shrubby, woody, and earthy material. The herbaceous and the shrub material, which included grass, leaves, hay, straw, fine hedge trimmings, lawn clippings, and small amounts of wood, was directly chopped and ensiled. It was reported that bacterial activity during the ensiling process promoted decomposition of the plant material. After ensiling, the samples underwent hydrothermal conditioning at temperatures between 40 and 60 °C. The objective of the study was to increase the methane yield of green waste and to improve the combustion properties of solid fuels from green waste press residues. It was reported that the calculated softening temperature of the ash (comparable to DT) was raised to 1,242 °C after conditioning at 40 °C (ratio 50:50 of herbaceous and shrub material) compared to the untreated raw material (1,053 °C). Finally, the authors provide the net energy yield of the so-called IFBB process at a conditioning temperature of 40 °C, which ranges from 1.96 to 2.85 kWh kg^{-1} DM^{-1} for methane yield and from 1.75 to 2.65 kWh kg^{-1} DM^{-1} for direct combustion.

Based on the study of Hensgen et al. (2011), the net energy yields of the developed thermo-biological pretreatment were also calculated for this thesis. Regarding the results from Chapter 4, the net energy yields range from 2.9 to 3.01 kWh kg^{-1} DM^{-1}. The aerobic configurations achieved a net energy yield of 2.9 kWh kg^{-1} DM^{-1} (SCDa-I) and 3.0 kWh kg^{-1} DM^{-1} (SCWa-I), and the anaerobic ones of 2.98 kWh kg^{-1} DM^{-1} (SCWa-B) and 3.01 kWh kg^{-1} DM^{-1} (SCDa-B).

Based on the results from Chapter 5, the net energy yields range from 3.96 to 4.5 kWh kg^{-1} DM^{-1} for direct combustion. Whereas the obtained net energy yields of the investigated configurations were below the raw material (4.45 kWh kg^{-1} DM^{-1}) and as discussed in Chapter 5, the observed drawbacks related to the calorific value can possibly be eliminated by adjustments of the straw–compost mixing ratios.

In Czekała (2021), the use of digestate as a solid fraction for the production of pellets as biofuel was investigated. Straw and sawdust additives were used in the production process of pellets. The observed calorific value, based on dry matter, for all pellets analyzed was between 5.32 to 5.52 kWh kg^{-1} DM^{-1}. However, no other combustion properties were investigated. Other studies have investigated the combination of anaerobic digestion and HTC to increase total bioenergy production (Aragón-Briceño, Ross, and Camargo-Valero 2021; Gaur et al. 2020; Parmar and Ross 2019; Reza et al. 2014). In addition to the use of lignocellulosic biomasses such as wheat straw, sewage sludge, and municipal solid waste in particular were investigated as feedstocks. The focus of these studies is the hydrothermal carbonization of digestate at different temperatures for HTC biochar production.

Biogas production from lignocellulosic biomass has several advantages compared to bioethanol production, as it has much higher overall energy efficiency in biogas production compared to ethanol (Shafiei et al. 2013). The advantage of this one-step process is simultaneous hydrolysis and biogas production, which in principle can be applied to all types of fermentable materials. The produced biogas can be used for electricity and heat generation or upgraded to gaseous fuels or biomethane (e.g., for the production of methanol, dimethyl ether, ammonia fuel, or platform chemicals), supporting the intended shift from fossil-based to bio-based industries (Shafiei et al. 2013; Moghaddam, Ahlgren, and Nordberg 2016). The integrated production of biogas and solid fuels has a positive impact on the environment, mainly by reducing the need for fossil fuels. Further research is needed to determine how the different (seasonal) compositions and qualities of the additive (compost) and wheat straw affect energy yields throughout the year.

6.4 Reflection and discussion of own approach

Straw qualities fluctuate over the year, and results may differ for other straw types. Long-term studies are therefore necessary to determine the range of variation. This also applies to the compost, which was subject to variations in its composition in the course of the thesis. A stockpile of materials was created, but a decrease in activity was noted. Accordingly, fresh compost material was always used for the results presented in this thesis. This may have led to different degradation rates of fibers and lignin.

After pretreatment, cellulose, hemicellulose, and lignin were degraded and partially converted into monosaccharides. Various methods were used to separate the fibers, e.g., centrifugal field, press screws, filtrations, and combinations of the mentioned methods. It was found that for laboratory applications the combination of membrane filter (pore size of 0.2 µm, *Interscience by Interlab*) and centrifuge (*Hermle*, Z 327 K) achieved the greatest separation efficiencies (calculated based on the TS contents according to VDI 3677; W=57% ± 9% after one minute at 12,500 rpm, W=75% ± 9% after 20 minutes at

12,500 rpm). For more reliable results, better comparison with other studies and, in terms of scale-up, vacuum filters would have been favorable (Wang et al. 2012; Raita et al. 2017; Trautmann and Krasny 2014; Mondylaksita et al. 2020; Sanchis-Sebastiá et al. 2020; J. A. Pérez et al. 2008; José A. Pérez et al. 2007; Manzanares et al. 2020; Isikgor and Becer 2015).

To verify and prove an increased bioconversion after pretreatment, the BMP was determined and compared. This demonstrated an increase in bioconversion of the wheat straw, taking into account the relevant literature. For detailed qualification of the microbial composition of the compost eluates, a powerful gene analytical Next Generation Sequencing (NGS) method should have been considered. Also, with regard to fluctuating compost compositions, qualities, and activities, precise determinations of the microorganisms (bacteria, archaea, fungi) could have been made. In the case of LD, determination of NH4-N by steam distillation would favor process adjustments with respect to the use of LD as an additive for pretreatment. This would have allowed more accurate descriptions as well as interpretations of the processes during the pretreatment period.

It would have been interesting to further investigate the leaching effects. The conclusion that alkali metals were removed came from the comparison of the washed and unwashed ash and thus served as a reference along with the review of previous literature. Elemental analysis would have been interesting as well; with knowledge of the C, H, N, S, O contents of the samples examined, the energy contents of straw, compost and LD, for example, could have been further specified and conclusions could possibly have been drawn about other ash-relevant components. In addition, the expanded chemical composition would have opened up further possibilities for interpreting the observed results in terms of both pretreatment and anaerobic digestion. For the combustion properties, the focus was on the three parameters calorific value, ash content, and melting temperatures. A thermogravimetric analysis would provide further insights, e.g., into oxidative mass losses due to the composition of the samples. In addition, emission measurements of the pellets produced would have underlined the value of solid fuels or identified new research approaches.

Allocation procedures should be avoided, if possible, since there are usually no optimal matching equivalence procedures (as in this thesis) and thus the result cannot represent the complete reality or the actual effects. A shortcoming of the crediting procedure is that the sum of emissions/resource consumptions of the investigated (extended) system may differ from the emissions/resource consumptions of the initial process (depending on the choice of reference processes). If, for example, product A and B are co-products and both product A and B can also be produced using alternative processes, the crediting procedure could be applied for A and B respectively. However, the sum of the emissions/resource consumptions from A and B would not necessarily correspond to the emissions/resource consumptions of the co-product. The inclusion of processes for recovering energy carriers from the fractions could have been a way out. However, this would have been beyond the scope of this thesis, as the primary objective was to develop and explore a pretreatment concept with a biological approach, taking into account cascade applications of additives.

Suitable software, such as *Aspen Plus*, is available for further development and optimization of the developed process setup. The energy and material flow models generated in most studies are based on simulations with *Aspen Plus* (Jeswani, Falano, and Azapagic 2015; Reynolds et al. 2016; Okolie et al. 2021; Vasconcelos et al. 2020; da Silva, Torres Ortega, and Rong 2016; D. Kumar and Murthy 2011; Harmsen et al. 1993; Julio et al. 2017; Voutilainen, Pihlajaniemi, and Parviainen 2021; Moghaddam, Ahlgren, and Nordberg 2016; Vasco-Correa and Shah 2019; Ingrao et al. 2021; Reynolds et al. 2016; Sassner, Galbe, and Zacchi 2008; Mandegari, Farzad, and Görgens 2018; Emeder 2021; Shafiei et al. 2013). A major handicap, however, is the high investment cost. Using the activity analysis approach, a techno-economic analysis can also be performed, but its quality is questionable because thermodynamic models cannot be implemented and numerous assumptions have to be made. *BioSTEAM* is an open-source platform designed to facilitate Techno-Economic Analysis (TEA) and Life-Cycle-Assessment (LCA) of biorefineries in thousands of scenarios. The example biorefineries are based on a biorefinery for corn straw developed with *Aspen Plus* (Humbird et al. 2011). As *BioSTEAM* is still under development, it is currently of limited use without the appropriate knowledge of the *Python* programming language. In the future, the selection of units must be increased to ensure easier operation. Up to now, many units, such as the pretreatment reactors or the fermenters, have to be defined in a very complex way for the respective problem. By using licensed and paid software, the casual user may get reliable results faster.

In order to improve the results of the present work, the mentioned adjustments as well as extensions of the research framework would be useful in the future.

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7 Conclusion and outlook

Towards circular economy, residual biomasses, e.g., wheat straw, are considered as an important sustainable raw material for the production of bioproducts and bioenergy. But, the high content of lignocellulose results in a limitation of the biodegradability of the straw. Direct use, e.g., as solid fuel, is also not recommended due to insufficient combustion properties. Therefore, appropriate pretreatments must be performed prior to bioenergy production. This thesis investigated how a combined thermo-biological pretreatment method using natural additives can improve both biodegradability and combustion properties.

Taking into account the existing material flows at the *:metabolon* research site, the effectiveness of green waste compost as a natural source of microorganisms for the degradation of lignocellulose, as well as the effectiveness of liquid digestate as a substitute for a lignocellulose-degrading ammonium solution, were investigated. It can be confirmed that thermophilic anaerobic pretreatment of lignocellulose from straw with compost releases fermentable sugars, resulting in a significant acceleration of methane production and an increase in methane yield. Furthermore, it can be confirmed that thermophilic anaerobic pretreatment reduces the ash content and improves the ash melting behavior. Thus, considering international standards, the use of wheat straw as a solid fuel would be possible. Furthermore, it was shown that green waste compost, as a component of the thermo-biological pretreatment process, is suitable as a natural source of microorganisms for the degradation of lignocellulose, and at the same time qualifies as a fuel additive and significantly improves the ash melting behavior of produced straw–compost pellets.

These practical findings, in pretreatment and both methane and pellet production, have made the bioconversion of straw lignocellulose more efficient. Consequently, its use as a feedstock in a biorefinery concept is more promising than before. The use of compost classifies the production system as a closed-loop and cascaded concept. Based on the findings in the field of straw fermentation, it was shown that the biological use of lignocellulose-degrading microorganisms enables an eco-friendly increase in substrate utilization, while at the same time expanding the substrate spectrum, which has long been discussed. The results of this research are significant because they provide an impetus for further development of the described methodology to establish an environmentally friendly and multi-stage cascaded biorefinery that processes multiple bio-based products from previously underutilized substrates such as lignocellulose from straw into higher value products. In addition, these results provide inspiration for the further development of environmentally friendly solid fuels based on straw–compost and contribute to establishing compost as a value-adding additive in several respects. Moreover, the developed methodology provides technological impulses for the further advancement of the straw market, especially in Germany, encouraging more efforts for the improvement of the regulatory market conditions in order to facilitate the realization of corresponding commercialization projects.

To demonstrate the technical applicability of a cascaded thermo-biological pretreatment method in a commercial-scale plant, the approaches presented here are relevant for future studies. In addition to the demonstrated environmental benefits, the yields and availabilities of the recovered lignocellulosic fractions are of critical importance for the establishment of a commercial biorefinery. Therefore, adjustment of the mixing ratios should identify an optimum that increases the methane yield and at the same time allows the development of an optimized standard fuel from the solid phase. If applicable, the advantages of the respective configurations investigated may be combined from a technical point of view. For example, due to the environmental advantages of the aerobic configurations, the incubation temperature of the anaerobic configuration could be lowered to 25 – 30 C° and the process temperature of the preceding thermal pressure hydrolysis increased, while maintaining the incubation time. Similarly, shortening the incubation time while increasing the temperature of the preceding thermal pressure hydrolysis could achieve a reduction in the total energy requirement and additionally increase the sugar yield.

Compared to the literature, the results have shown that combined biological-(hydro)thermal processes are well suited to improve both bioconversion in fermentative processes and fuel properties of waste streams from lignocellulosic feedstocks such as wheat straw. For large-scale applications of the combined biological pretreatment shown in this work, HTC seems to be advantageous from a technical point of view. In general, it can be concluded that compost causes the degradation of lignocellulose and increases bioconversion. Future research should therefore investigate different composts for effective microbial strains in different seasons to achieve the maximum degradation rates. This will also build the necessary know-how to contribute in the further development of "home-grown" highly active enzymes. In the case of liquid digestate, another "home-grown" additive, the effects of $NH4^+$-induced ammonolysis need to be further explored to promote liquid digestate for synergistic pretreatment of lignocellulosic material. Process- and cascade-optimized thermo-biological pretreatments appear to be best suited to meet the challenges of a circular economy, as they are both the least harmful and the most sustainable in terms of ecology.

The technological and ecological analyses carried out illustrate the potential of the studied pretreatment concept for a biorefinery. This thesis contributes to the basic research for the development of environmentally friendly and circular biorefinery concepts. In particular, the use of biological pretreatment of biomass was highlighted. However, the transfer of the investigated biorefinery concept from the research stage to the pilot and full-scale production stage requires a distinctly high degree of technological maturity for implementation on a greenfield site. Nevertheless, it will still require a high level of investment for construction and operation. Considering the current situation of biomass utilization, the conversion and retrofitting of suitable biomass-conversion-plants, e.g., landfills with onsite composting and biogas plant, into biorefineries with integrated material and energy utilization of renewable raw materials seems to be a promising future scenario.

8 Summary

Dependence on resource-rich countries combined with geopolitical risks and, in particular, climate change increases the need for research on developing processes for using biomass as an alternative raw material, both in the energy industry and in the chemical industry. Therefore, several bioeconomy strategies have been developed and biorefinery process development has been financially supported in the last decade – in Europe and worldwide. As a technology-oriented country, Germany is particularly dependent on innovative sources of raw materials. This includes the use of materials and energy, especially lignocellulosic biomasses which are not in competition with the food industry. Lignocellulose is the main component of the plant cell wall and consists of the sugar polymers hemicellulose and cellulose and the aromatic polymer lignin. Similar to petroleum, lignocellulose has a complex composition and in order to make the polymers usable, the raw material must first be fractionated into its chemical components.

This thesis investigated whether the utilization efficiency of wheat straw can be increased by novel thermo-biological process combinations. The overall objective was the development of an eco-friendly pretreatment process for lignocellulosic straw incorporating natural additives in a utilization cascade, improving both biodegradability and combustion properties of the feedstock. Therefore, the focus of this thesis was the utilization of wheat straw in a combined process of biogas and solid fuel production. In addition to the technical development, an accompanying techno-ecological evaluation of the processes developed in a laboratory scale was carried out.

In Germany, cereal straw, and specifically wheat straw, has the highest mobilization potential among renewable feedstocks, at 4 to 9 million tons per year (corresponding to an energy potential of 57 to 129 PJ/a). Hence, wheat straw is of major interest in Germany, as a sustainable and efficient use of straw contributes to saving fossil raw materials. For this reason, various methods for pretreatment of lignocellulosic straw have been investigated and occasionally combined in recent years. Based on the state of the art and the respective advantages and disadvantages of the different pretreatment methods, two reference methods were selected which are considered most favorable from a technical point of view (acetone-based Organo-Solv method) and from an ecological point of view (Liquid-Hot-Water method). Thus, a comparative framework was established to rank the performance of the developed (combined) method in the overall context of pretreatment methods according to relevant criteria.

The high content of lignocellulose results in a limitation of biodegradability of wheat straw. For this reason, suitable pretreatments for optimized use in bioenergy production are required. Thermo-biological pretreatment methods with different process parameters were investigated and compared. In this regard, the effect of additives such as liquid digestate and green waste compost, which are capable of degrading lignocellulosic materials first and then digesting them for methane production, were of particular interest. Mechanically comminuted wheat straw was mixed with water or liquid digestate to achieve a dry matter content of 30%. Thermal treatment of the samples was performed by autoclave (120 – 140 °C, 20 min). Subsequently, autoclaved substrate was mixed with compost from the hot rotting phase and incubated under mesophilic (25 °C) aerobic and thermophilic (50 °C) anaerobic conditions for 14 days each. In order to compare the effects of substrate decomposition into the fractions cellulose, hemicellulose, and lignin after pretreatment, the respective substrate compositions were analyzed to determine the lignocellulose fractions using a high-performance liquid chromatograph. An improvement of the biodegradability of the lignocellulosic materials by adding compost as a biological pretreatment was observed. Biomethane potential was investigated on a laboratory scale in batch tests according to VDI 4630. Methane production of the pretreated samples ranged from 270 to 320 L kg^{-1} oTS^{-1}. The use of compost as a natural source of microorganisms degrading lignocellulosic biomasses had a beneficial effect on the methane production of the samples. The addition of compost reduced technical digestion time by 35 to 91% and increased methane yield up to 32% compared to untreated straw. More than 84% of methane were already produced during thermophilic anaerobic biological pretreatment and captured due to the anaerobic configuration, minimizing metabolic losses compared to the aerobic configuration.

Besides limited biodegradability, in the literature straw is also known for its inferior combustion properties, such as low calorific value, high ash content, and low ash melting temperature, which complicates its use as a solid fuel. Compost has not yet been considered as an additive. Therefore, the combustion properties (calorific value according to ISO 18125, ash content according to ISO 18122, and ash melting behavior according to ISO 21404) of dewatered solid mixtures in pellet form were investigated after the described thermo-biological pretreatment approaches. By means of analysis of variance (ANOVA), improvements in the calorific value and ash melting behavior of the thermophilic anaerobically pretreated samples were verified. Although melting temperatures were significantly increased (shrinkage start temperature up to 659 °C higher compared to untreated straw), adverse effects on ash content (increased) and heating value (decreased) occurred due to the homogeneous straw–compost mixture. These adverse effects were reduced by autoclave treatment at 140 °C in conjunction with the dewatering process (leaching of alkali metals). However, the fuel specification ISO 17225-6 was not met for the solid mixtures. Nevertheless, the potential of compost is promising and qualifies this material as a possible additive to optimize the ash melting behavior of straw pellets.

Within the scope of a Life-Cycle-Assessment according to ISO 14040/14044, the reference processes established in the literature for the pretreatment of wheat straw and the developed thermo-biological approaches were examined for their respective environmental impacts. For both the reference processes and the pretreatment concepts developed in this thesis, all relevant mass flows, auxiliary materials, and energy requirements were classified. The *ReCiPe 2016 v1.1 midpoint (H)* method and the impact factors of global warming potential, human toxicity, freshwater eutrophication, freshwater toxicity,

terrestrial acidification, and fossil degradation were chosen to calculate the main environmental impact factors of the studied pretreatment methods. Due to the quantitative and qualitative differences of the fractionated lignocellulosic components, the reference per kilogram dry matter (DM) or the dimension 1 kg DM-straw was used as the functional unit for the life cycle inventory. On this basis, a comparative framework with weighted criteria following the guideline VDI 2225 was developed, which allowed a techno-ecological evaluation of the pretreatment methods, thus determining potential environmental impacts and identifying shortcomings in the processes. Compared to the selected reference processes, the developed configurations performed favorably in terms of both environmental and technical criteria. In addition, the results confirmed that the Liquid-Hot-Water method, which uses water as a solvent at temperatures above 200 °C (at high pressure), is more environmentally friendly than the acetone-based method. However, as a result of the techno-ecological comparison, the technical advantages of the acetone-based treatment were elaborated: enhanced separation of the fractions, resulting in better quality as well as higher accessibility of the fractions, especially lignin. The Liquid-Hot-Water method achieved a better mass balance of the cellulose and hemicellulose fractions and was characterized by low environmental impact potentials. Overall, the use of biological pretreatment in combination with preceding thermal pressure hydrolysis (in the low-pressure range) had a major advantage over the chemical pretreatment method in particular, since the additives used (compost or liquid digestate) led to ecological savings in the Life-Cycle-Assessment.

In conclusion, the cascaded use of straw–compost mixtures increases the value-added opportunities of the biorefinery concept and leads to a better utilization of feedstocks by optimized production of gaseous and solid energy sources. Based on this thesis, a process- and cascade-optimized thermo-biological pretreatment process may favor the transformation of landfills with connected composting and biogas plants into biorefineries with integrated material and energetic use of renewable resources, enabling an economic feasibility of a lignocellulosic biorefinery.

9 Zusammenfassung

Abhängigkeiten von rohstoffreichen Ländern verbunden mit geopolitischen Risiken und insbesondere dem Klimawandel erhöhen den Forschungsbedarf in der Entwicklung von Verfahren zur Nutzung von Biomasse als alternativem Rohstoff sowohl in der Energiewirtschaft als auch in der chemischen Industrie. In den letzten zehn Jahren wurden daher sowohl in Europa als auch weltweit mehrere Bioökonomie-Strategien entwickelt und die Bioraffinerieprozessentwicklung finanziell gefördert. Deutschland als technologieorientiertes Land ist in besonderem Maße auf innovative Rohstoffquellen angewiesen. Hierzu gehören die stoffliche und die energetische Nutzung insbesondere lignocellulosehaltiger Biomassen, die nicht in Konkurrenz mit der Nahrungsmittelindustrie stehen. Lignocellulose ist der Hauptbestandteil der pflanzlichen Zellwand und besteht aus den Zuckerpolymeren Hemicellulose und Cellulose sowie dem aromatischen Polymer Lignin. Ähnlich wie Erdöl hat Lignocellulose eine komplexe Zusammensetzung. Um die Polymere nutzbar zu machen, muss das Rohmaterial vorher in seine chemischen Bestandteile fraktioniert werden.

In dieser Thesis wurde untersucht, ob die Verwertungseffizienz von Weizenstroh durch neuartige thermo-biologische Verfahrenskombinationen gesteigert werden kann. Das übergeordnete Ziel war die Entwicklung eines umweltfreundlichen Vorbehandlungsverfahrens für Lignocellulose, welches natürliche Zusatzstoffe in einer Nutzungskaskade einbindet, wodurch die biologische Abbaubarkeit und die Verbrennungseigenschaften des Ausgangsstoffs verbessert werden. Im Mittelpunkt dieser Arbeit stand daher die Nutzung von Weizenstroh in einem kombinierten Prozess der Biogas- und Festbrennstoffproduktion. Neben der technischen Entwicklung wurde auch eine begleitende technisch-ökologische Bewertung der im Labormaßstab entwickelten Verfahren durchgeführt.

In Deutschland hat Getreidestroh, speziell Weizenstroh, mit 4 bis 9 Millionen Tonnen pro Jahr (das entspricht einem Energiepotenzial von 57 bis 129 PJ/a) das höchste Mobilisierungspotenzial unter den erneuerbaren Rohstoffen. Dementsprechend ist Weizenstroh in Deutschland von besonderem Interesse, da eine nachhaltige und effiziente Nutzung des Strohs zur Einsparung von fossilen Rohstoffen beitragen kann. Aus diesem Grund wurden in den letzten Jahren verschiedene Methoden zur Vorbehandlung von Lignocellulose untersucht und teilweise kombiniert. Ausgehend vom aktuellen Stand der Technik und den jeweiligen Vor- und Nachteilen der verschiedenen Vorbehandlungsmethoden wurden zwei Referenzmethoden ausgewählt, die zum einem aus technischer Sicht (Aceton-basierte Organo-Solv Methode) und zum anderem aus ökologischer Sicht (Liquid-Hot-Water Methode) besonders vorteilhaft anzusehen sind. Somit wurde ein Vergleichsrahmen geschaffen, um die Performance des entwickelten (kombinierten) Verfahrens im Gesamtkontext der Vorbehandlungsverfahren nach relevanten Kriterien einordnen zu können.

Der hohe Gehalt an Lignocellulose hat eine Einschränkung der biologischen Abbaubarkeit des Strohs zur Folge. Daher müssen geeignete Vorbehandlungen von Weizenstroh für den optimierten Einsatz in der Bioenergieproduktion durchgeführt werden. Es wurden thermo-biologische Vorbehandlungsmethoden mit unterschiedlichen Prozessparametern untersucht und verglichen. Dabei war der Effekt von Additiven wie flüssiger Gärrest und Grüngutkompost, die Lignocellulosematerialien zunächst aufschließen und dann zur Methanproduktion abbauen können, von besonderem Interesse. Mechanisch zerkleinertes Weizenstroh wurde mit Wasser (oder flüssigem Gärrest) angemaischt, um einen Trockensubstanzgehalt von 30% einzustellen. Die thermische Vorbehandlung der Proben erfolgte mittels Autoklav (120 – 140 °C, 20 min). Anschließend wurde autoklaviertes Substrat mit Kompost aus der Heißrottenphase vermischt und unter mesophil (25 °C) aeroben und thermophil (50 °C) anaeroben Bedingungen für jeweils 14 Tage inkubiert. Um die Effekte des Substrataufschlusses in die Fraktionen Cellulose, Hemicellulose und Lignin nach der Vorbehandlung miteinander vergleichen zu können, wurden die jeweiligen Substratzusammensetzungen zur Bestimmung der Lignocelluloseanteile mittels Hochleistungs-Flüssigkeitschromatographen analysiert. Eine Verbesserung der biologischen Abbaubarkeit der lignocellulosehaltigen Materialien durch die Zugabe von Kompost als biologische Vorbehandlung konnte beobachtet werden. Das Biomethanpotenzial wurde im Labormaßstab in Batch-Versuchen nach VDI 4630 untersucht. Die Methanproduktion der vorbehandelten Proben bewegte sich zwischen 270 und 320 L kg^{-1} oTS^{-1}. Die Verwendung von Kompost als natürliche Quelle von Mikroorganismen, die lignocellulosehaltige Biomassen abbauen, wirkte sich günstig auf die Methanproduktion der Proben aus. Die Zugabe von Kompost führte zu einer um 35 bis 91% verkürzten technischen Vergärungszeit und einer um bis zu 32% gesteigerten Methanausbeute im Vergleich zu unbehandeltem Stroh. Mehr als 84% des Methans wurden bereits während der thermophilen anaeroben biologischen Vorbehandlung gebildet und aufgrund der anaeroben Konfiguration aufgefangen, wodurch Stoffwechselverluste im Vergleich zur aeroben Konfiguration minimiert wurden.

Neben der eingeschränkten biologischen Abbaubarkeit ist Stroh in der Literatur auch für seine minderwertigen Verbrennungseigenschaften bekannt, wie z.B. den niedrigen Heizwert, den hohen Aschegehalt und die niedrige Schmelztemperatur der Asche, was seine Verwendung als Festbrennstoff erschwert. Kompost wurde bisher noch nicht als Additiv in Betracht gezogen. Daher wurden die Verbrennungseigenschaften (Heizwert nach ISO 18125, Aschegehalt nach ISO 18122 und Ascheschmelzverhalten nach ISO 21404) von entwässerten Feststoff-Mischungen in Pelletform nach den beschriebenen thermo-biologischen Vorbehandlungsansätzen untersucht. Mittels Varianzanalyse (ANOVA) konnten Verbesserungen des Heizwerts und des Ascheschmelzverhaltens der thermophil anaerob vorbehandelten Proben nachgewiesen werden. Obwohl die Schmelztemperaturen deutlich erhöht wurden (Schrumpfungsstarttemperatur um bis zu 659 °C höher im Vergleich zu unbehandeltem Stroh), traten aufgrund der homogenen Stroh-Kompost-Mischung negative Auswirkungen auf den Aschegehalt (erhöht) und den Heizwert (verringert) auf. Diese negativen Auswirkungen konnten durch die Autoklav-Behandlung bei 140 °C in Verbindung mit dem Entwässerungsprozess (Auswaschen von Alkalimetallen) reduziert werden. Die Brennstoffspezifikation ISO 17225-6 wurde für die Feststoff-Mischungen allerdings nicht erfüllt. Das Potenzial von Kompost ist jedoch vielversprechend und qualifiziert dieses Material als möglichen Zusatzstoff, um das Ascheschmelzverhalten von Stroh-Pellets zu optimieren.

Im Rahmen einer Ökobilanz nach ISO 14040/14044 wurden die in der Literatur etablierten Referenzprozesse zur Vorbehandlung von Weizenstroh und die entwickelten thermo-biologischen Ansätze auf ihre jeweiligen Umwelteinflüsse hin untersucht. Sowohl für die Referenzprozesse als auch für die in dieser Thesis entwickelten Vorbehandlungskonzepte wurden alle relevanten Massenströme, Hilfsstoffe und Energiebedarfe klassifiziert. Zur Berechnung der wichtigsten Umweltwirkungsfaktoren der untersuchten Vorbehandlungsmethoden wurden die Methode *ReCiPe 2016 v1.1 midpoint (H)* und die Wirkungsfaktoren Treibhauspotenzial, Humantoxizität, Süßwasser-Eutrophierung, Süßwasser-Toxizität, terrestrische Versauerung und fossiler Abbau gewählt. Aufgrund der quantitativen und qualitativen Unterschiede der fraktionierten lignocellulosehaltigen Bestandteile wurde als funktionelle Einheit für die Sachbilanz der Bezug pro Kilogramm Trockenmasse (TS) bzw. die Dimension 1 kg TS-Weizenstroh verwendet. Darauf aufbauend wurde ein Vergleichs-Framework mit gewichteten Kriterien in Anlehnung an die Richtlinie VDI 2225 entwickelt, der eine technisch-ökologische Bewertung der Vorbehandlungsmethoden ermöglichte, wodurch potenzielle Umweltauswirkungen ermittelt und Schwachstellen in den Verfahren identifiziert werden konnten. Im Vergleich zu den ausgewählten Referenzverfahren zeigten die entwickelten Konfigurationen sowohl in Bezug auf ökologische als auch auf technische Kriterien eine gute Performance. Zudem haben die Ergebnisse bestätigt, dass die Liquid-Hot-Water Methode, welche Wasser als Lösungsmittel bei Temperaturen über 200 °C verwendet, umweltfreundlicher als das aceton-basierte Verfahren ist. Anhand des techno-ökologischen Vergleichs wurden jedoch die technischen Vorteile der aceton-basierten Behandlung herausgearbeitet: Die bessere Trennung der Fraktionen, wodurch eine höhere Qualität und vor allem eine bessere Zugänglichkeit der Ligninfraktionen erreicht wurden. Die Liquid-Hot-Water Methode erzielte eine bessere Massenbilanz der Cellulose- und Hemicellulose-Fraktionen und zeichnete sich durch geringe Umweltwirkungspotenziale aus. Insgesamt hatte der Einsatz der biologischen Vorbehandlungen in Kombination mit der vorgeschalteten thermischen Druckhydrolyse (im Niederdruckbereich) insbesondere gegenüber der chemischen Vorbehandlungsmethode einen großen Vorteil, da die eingesetzten Zusatzstoffe (Kompost bzw. Flüssiggärrest) zu ökologischen Einsparungen in der Ökobilanz führten.

Abschließend lässt sich festhalten, dass der kaskadierte Einsatz von Stroh-Kompost-Gemischen die Wertschöpfungsmöglichkeiten des Bioraffineriekonzepts erhöht und zu einer besseren Verwertung der Einsatzstoffe durch optimierte Produktion von gasförmigen und festen Energieträgern führt. Basierend auf den Ergebnissen dieser Thesis könnte ein prozess- und kaskadenoptimiertes thermo-biologisches Vorbehandlungsverfahren die Umwandlung von Deponien mit angeschlossener Kompostierung und Biogasanlage in Bioraffinerien mit integrierter stofflichen und energetischen Nutzung nachwachsender Rohstoffe begünstigen und dadurch die wirtschaftliche Machbarkeit einer Lignocellulose-Bioraffinerie ermöglichen.

Appendix

The supplementary data to Chapter 5 can be found on the data storage medium enclosed to this thesis and online:

https://ars.els-cdn.com/content/image/1-s2.0-S001623612202662X-mmc1.pdf

Table 24: Life cycle inventory data of the thermal-biological pretreatment methods (SCWa-I; SCDa-I; SCWa-B; SCDa- B).

Process module	Einheit	Vorbehandlungsmethode				Energy flow / Elemental flow	Input/Output
		SCWa-I	SCWa-B	SCDa-I	SCDa-B		
Fuel consumption transport	kg/kgS(DM)	0,00033	0,00033	0,00033	0,00033	Elemental flow	Input
Moisture export storage	kg/kgS(DM)	0,068	0,068	0,068	0,068	Elemental flow	Output
Energy input mech. pretreatment	kWh/kgS(DM)	0,19756	0,19756	0,19756	0,19756	Energy flow	Input
Straw mass	kg/kgS(DM)	1,07759	1,07759	1,07759	1,07759	Elemental flow	Input
Mass of water/liquid digestate	kg/kgS(DM)	2,25575	2,25575	2,94227	2,94227	Elemental flow	Input
Energy supply Mixture	kWh/kgS(DM)	0,04444	0,04444	0,05359	0,05359	Energy flow	Input
Energy input thermal pretreatment	kWh/kgS(DM)	0,314	0,314	0,37647	0,37647	Energy flow	Input
Waste heat export thermal pretreatment	kWh/kgS(DM)	0,2	0,2	0,2	0,2	Energy flow	Output
Mass of compost biological pretreatment	kg/kgS(DM)	2,899	2,899	2,899	2,899	Elemental flow	Input
Energy supply mixture biological pretreatment	kWh/kgS(DM)	0,08310	0,08310	0,09225	0,09225	Energy flow	Input
Energy supply incubator	kWh/kgS(DM)	6,74037	60,06079	6,74347	60,13414	Energy flow	Input
Waste heat incubator	kWh/kgS(DM)	6,72	59,64	6,72	59,64	Energy flow	Output
Energy supply separation	kWh/kgS(DM)	6,04119	6,04119	6,6966	6,6966	Energy flow	Input
Mass of solid fraction	kg/kgS(DM)	4,986	4,986	5,535	5,535	Elemental flow	Output
Mass of liquid fraction	kg/kgS(DM)	1,24647	1,24647	1,38377	1,38377	Elemental flow	Output

Table 25: Overall summary including environmental credits of the techno-ecological comparison.

			Weighting						Raw data of conversion rates						Unit
			Thermo-biological				Physical-chemical (Hydrothermal)	Chemical (organic solvent)	Thermo-biological				Physical-chemical (Hydrothermal)	Chemical (organic solvent)	
			SCWa-I	SCWa-B*	SCDa-I	SCDa-B*	LHW	Aceto-Solv	SCWa-I	SCWa-B*	SCDa-I	SCDa-B*	LHW	Aceto-Solv	
Technical Criteria	**Quantity Glucose**	**10%**	80%	78%	70%	38%	100%	78%	334	325	292	159	417	325	g / kg DM
	Availability Glucose	**6%**	50%	50%	50%	50%	75%	100%	2	2	2	2	3	4	(-)
	Quantity Xylose	**7%**	78%	75%	100%	44%	27%	27%	172	164	220	97	59	60	g / kg DM
	Quantity Arabinose	**7%**	68%	68%	68%	69%	34%	28%	34	33	50	35	17	14	g / kg DM
	Availability Xylose, Arabinose	**6%**	40%	60%	40%	60%	100%	80%	2	3	2	3	5	4	(-)
	Quantity Lignin	**4%**	72%	82%	58%	100%	36%	29%	400	452	321	554	199	161	g / kg DM
	Availability Lignin	**5%**	20%	40%	20%	40%	60%	100%	1	2	1	2	3	5	(-)
Ecological Criteria (incl. Environmental credits)	**Global Warming Potential**	**15%**	89%	16%	100%	16%	37%	7%	5.39	29.71	4.79	29.18	12.8	67.4	kg CO2-equiv. / kg DM
	Freshwater Eutrophication	**10%**	100%	73%	100%	73%	-89%	-2459%	-5.51E-05	-4.04E-05	-5.53E-05	-4.05E-05	4.91E-05	1.36E-03	kg P-equiv. / kg DM
	Freshwater Toxicity	**5%**	96%	12%	89%	12%	100%	2%	9.10E-04	7.39E-03	9.77E-04	7.46E-03	8.72E-04	5.22E-02	kg 1.4-DCB-equiv. / kg DM
	Human Toxicity	**5%**	98%	9%	100%	9%	22%	1%	0.06	0.63	0.06	0.63	0.249	8.58	kg 1.4-DCB-equiv. / kg DM
	Terrestrial Acidification	**10%**	34%	28%	63%	26%	100%	20%	5.22E-02	6.33E-02	2.82E-02	6.68E-02	1.77E-02	8.73E-02	kg SO2-equiv. / kg DM
	Fossil Degradation	**10%**	83%	13%	100%	13%	21%	3%	1.36	8.77	1.13	8.55	5.29	37.1	kg oil-equiv. / kg DM
		Results	**72%**	**45%**	**78%**	**39%**	**44%**	**-214%**							

*Partial conversion of sugars to methane during anaerobic incubation.

Table 26: Overall summary excluding environmental credits of the techno-ecological comparison.

			Weighting						Raw data of conversion rates						Unit
			Thermo-biological				Physical-chemical (Hydrothermal)	Chemical (organic solvent)	Thermo-biological				Physical-chemical (Hydrothermal)	Chemical (organic solvent)	
			SCWa-I	SCWa-B*	SCDa-I	SCDa-B*	LHW	Aceto-Solv	SCWa-I	SCWa-B*	SCDa-I	SCDa-B*	LHW	Aceto-Solv	
Technical Criteria	Quantity Glucose	10%	80%	78%	70%	38%	100%	78%	334	325	292	159	417	325	g / kg DM
	Availability Glucose	6%	50%	50%	50%	50%	75%	100%	2	2	2	2	3	4	(-)
	Quantity Xylose	7%	78%	75%	100%	44%	27%	27%	172	164	220	97	59	60	g / kg DM
	Quantity Arabinose	7%	68%	68%	68%	69%	34%	28%	34	33	50	35	17	14	g / kg DM
	Availability Xylose, Arabinose	6%	40%	60%	40%	60%	100%	80%	2	3	2	3	5	4	(-)
	Quantity Lignin	4%	72%	82%	58%	100%	36%	29%	400	452	321	554	199	161	g / kg DM
	Availability Lignin	5%	20%	40%	20%	40%	60%	100%	1	2	1	2	3	5	(-)
Ecological Criteria (excl. Environmental credits)	Global Warming Potential	15%	100%	21%	95%	21%	51%	10%	6.58	30.9	6.91	31.3	12.8	67.4	kg CO2-equiv. / kg DM
	Freshwater Eutrophication	10%	100%	76%	100%	75%	92%	3%	4.53E-05	6.00E-05	4.54E-05	6.02E-05	4.91E-05	1.36E-03	kg P-equiv. / kg DM
	Freshwater Toxicity	5%	43%	10%	41%	10%	100%	2%	2.03E-03	8.51E-03	2.12E-03	8.60E-03	8.72E-04	5.22E-02	kg 1.4-DCB-equiv. / kg DM
	Human Toxicity	5%	100%	26%	94%	25%	57%	2%	0.141	0.549	0.15	0.559	0.249	8.58	kg 1.4-DCB-equiv. / kg DM
	Terrestrial Acidification	10%	32%	27%	56%	25%	100%	20%	5.45E-02	6.56E-02	3.15E-02	7.01E-02	1.77E-02	8.73E-02	kg SO2-equiv. / kg DM
	Fossil Degradation	10%	100%	19%	95%	19%	33%	5%	1.77	9.18	1.87	9.29	5.29	37.1	kg oil-equiv. / kg DM
		Results	**73%**	**47%**	**74%**	**41%**	**67%**	**33%**							

*Partial conversion of sugars to methane during anaerobic incubation.

$$\text{solid recovery (\%)} = \frac{W_{\text{pretreated}}}{W_{\text{untreated}}} \times 100\%$$

$$\text{glucan recovery (\%)} = \frac{W_{\text{pretreated}} \times \text{glucan content after pretreatment}}{W_{\text{untreated}} \times \text{glucan content in untreated}} \times 100\%$$

$$\text{xylan recovery (\%)} = \frac{W_{\text{pretreated}} \times \text{xylan content after pretreatment}}{W_{\text{untreated}} \times \text{xylan content in untreated}} \times 100\%$$

Figure 26: Solid recovery, glucan recovery and xylan recovery (Zhang et al. 2022).