Universität für Bodenkultur Wien
University of Natural Resources
and Applied Life Sciences, Vienna

Department of Agrobiotechnology, IFA-Tulln

Institute of Environmental Biotechnology

Recovering polyethylene terephthalate from cotton/PET blends: a circular economy concept for textile waste

Master Thesis

Supervisor

Univ.Prof. Dipl.-Ing. Dr. Georg Gübitz

Felice Quartinello, M.Sc.

Submitted by

Sebastian Gritsch, B.Sc.

Vienna, October 2019

Statutory declaration

I hereby declare that I am the sole author of this work. No assistance other than that which is permitted has been used. Ideas and quotes taken directly or indirectly from other sources are identified as such. This written work has not yet been submitted in any part.

Vienna, October 2019

Sebastian Gritsch

Abstract

While the textile industry is ever-growing and more clothes and textiles are consumed across the globe, there are still no strategies for recycling these materials. The largest share of textiles is incinerated or deposited in landfills, even though they could be used for the production of new materials.

The composition of modern fibres poses a challenge for the recycling process. In order to benefit from properties of individual materials, textiles are increasingly made from mixed fibres. One of the most widely used combinations of natural and synthetic fibres are cotton-polyester blends, which combine good water absorption, the pleasant touch and robustness of their components. While recycling of pure fibres is simple, these mixtures have to be separated before the individual materials can be recycled efficiently.

In this thesis an enzymatic approach for the separation of cotton-polyester blends was investigated. The cotton fraction was hydrolysed to glucose by cellulases allowing the recovery of polyethylene terephthalate (PET) which was successfully spun into new fibres.

To accelerate the process, various pre-treatment methods like ultrasound, alkaline treatment and steam explosion as well as the effects sample size, mass transfer and the solvent Cyrene have on the hydrolysis were investigated.

The combination of steam explosion and alkaline pre-treatment followed by a 7-day hydrolysis resulted in a highly pure PET fraction, which was confirmed via FTIR measurements. The addition of 1 % Cyrene significantly improved the hydrolysis, leading to higher a glucose concentration and weight loss of the fibres.

The recovered glucose was successfully used as carbon source in the production of bio-ethanol from *Saccharomyces cerevisiae* as well as cellulose degrading enzymes from *Trichoderma reesei*.

Kurzfassung

Während die Textilindustrie weiter wächst und weltweit immer mehr Gewand und Textilien konsumiert werden gibt es immer noch keine Strategie für die Wiederverwertung dieser Stoffe. Der größte Anteil an Textilien findet früher oder später seine Bestimmung in Müllverbrennungen oder Deponien. Dabei bieten sie wertvolle Rohstoffe, die wieder in die Produktion neuer Fasern einfließen könnten.

Die Zusammensetzung moderner Textilien stellt diesbezüglich eine Herausforderung dar. Um von den individuellen Eigenschaften einzelner Fasern zu profitieren, werden Textilien zunehmend aus gemischten Stoffen hergestellt. Eine der verbreitetsten Kombinationen sind Baumwoll-Polyester-Gemische, welche die Wasseraufnahmefähigkeit, Robustheit und das angenehme Tragegefühl ihrer Komponenten vereinen.

Eine Wiederverwertung reiner Fasern ist aus technischer Sicht einfach, jedoch müssen Gemische erst aufgetrennt werden, bevor die einzelnen Stoffe wiederverwendet werden können. In dieser Arbeit wurde die enzymatische Trennung von Baumwoll-Polyester-Gemischen zur Gewinnung einer reinen Polyesterfraktion untersucht. Dabei wurde der Baumwollanteil mithilfe von Cellulasen zu Glucose hydrolysiert und das zurückbleibende Polyethylentherephthalat (PET) erfolgreich zu neuen Fasern gesponnen.

Zur Beschleunigung des Prozesses wurden Vorbehandlungen mit Ultraschall, Natronlauge und Steam-explosion sowie die Einflüsse von Probengröße, Massentransfer und dem Lösungsmittel Cyrene auf die Hydrolyse untersucht. Durch die Kombination von Steam-explosion und alkalischer Vorbehandlung konnten nach anschließender enzymatischer Hydrolyse reines PET gewonnen werden, was mittels FTIR Messungen bestätigt wurde. Die Zugabe von 1 % Cyrene während der Hydrolyse begünstigte den Celluloseabbau, was sich durch eine höhere Glucosekonzentration und einen höheren Gewichtsverlust der Fasern bemerkbar machte.

In ersten Fermentationen wurden aus der anfallenden Glucose Bioethanol sowie neue Enzyme für den Abbau von Cellulose hergestellt.

Acknowledgements

I want to thank my supervisor Univ.Prof. Dipl.-Ing. Dr. Georg Gübitz for giving me the opportunity to work at the Institute of Environmental Biotechnology. Furthermore, I want to thank my co-supervisor Felice Quartinello, M.Sc. for a very kind welcome to the working group and for his guidance during my work on this thesis.

Special thanks go to everyone at the institute. The team provided a warm working environment and always offered a helping hand when needed.

Finally, I want to express my deep gratitude to my family and friends for their support and encouragement. Without their help I would never have come this far.

List of figures

List of tables

List of equations

Table of contents

1 Introduction

1.1 Textile waste situation

Even though most of today's clothing is produced in Asia, the textile and clothing industry still has a large market in Europe. In 2017 the industry had a turnover of more than 150 billion euros employing over 1.5 million people (Statistical Office of the European Communities, 2019c). The contribution of expenses for clothing to total household consumption in 1996 was 5 %. This number hardly changed to 2018, where the share dropped to 4 % of household expenses (Statistical Office of the European Communities, 2019b). Contrary to what this decrease suggests, people did not acquire less clothing. When changes in pricing are taken into account the total amount of clothes purchased increased by 40 % in the period between 1996 to 2012 according to an European Environmental Agency report (Reichel, Mortensen, Asquith, & Bogdanovic, 2014).

Referring to Fast Food, this phenomenon is called "Fast Fashion". Trying to capture fashion trends from the catwalk and quickly delivering them to customers at cheap prices the textile industry created an environmental challenge. The average number of fashion collections each year has increased drastically. Companies like H&M and Zara now offer between 12 and 24 collections per year, while in 2011 there were just five (Remy, Speelman, & Swartz, 2016). Consumers more often loose a sense of value for clothing and exchange their wardrobe more frequently, either because it is "out of fashion" or damaged, as similar to Fast Food the materials used for these products most often are of lower quality (Amed et al., 2017).

In 2007 the total consumption of textiles, including clothing and household textiles like bed sheets and carpets, in the EU was 9.55 million tonnes (Beton et al., 2014). A more recent estimation of the Waste and Resources Action Programme (WRAP UK) mentions 6.4 million tonnes of clothing in the EU in 2015 (Gray, 2017). At the end of their use more than 50 % of European textiles were disposed of through the mixed household waste eventually being landfilled or incinerated (Beton et al., 2014). While the data used in this article is from 2004 more recent studies indicate, though there is a large variance between countries, that the overall situation in the EU did not change much till today. On average only 36 % of textiles are collected separately. Germany has an exceptionally high share with 75 %, while in Italy only 11 % of clothes are collected separately (Watson, Aare, Trzepacz, & Peterse, 2018).

A total of 738 billion tonnes of waste were treated within the EU in 2016, whereof 57 % were recycled, 24 % were landfilled and 18 % were incinerated or used for energy recovery.

There are big differences in waste treatment among EU member states as can be seen in Figure 1.

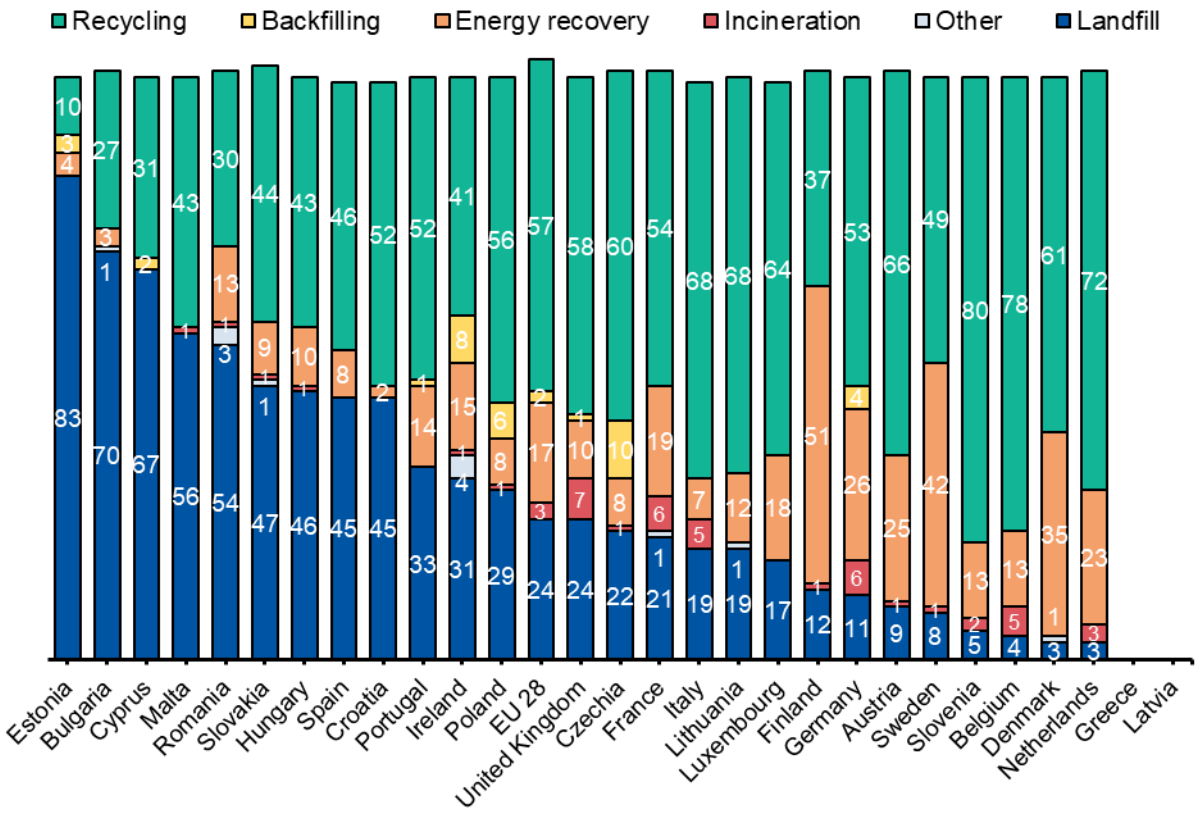

Figure 1: Waste treatment within EU member states in 2016. Data for incineration (GER, AUT), energy recovery (GER, AUT) and recycling (GER) were supplemented from 2014 statistics as data from 2016 is not available. Data from Greece and Latvia is not available (reference Waste Treatment in EU).

Landfilling and incineration are least preferred of these waste treatment methods as many valuable resources and energy are thrown away. During incineration and energy recovery waste is combusted. The difference, as the name suggests, is that during combustion in Waste to Energy (WTE) plants energy is recovered in the form of heat and electrical energy, while during incineration no energy recovery takes place. Therefore, WTE plants are preferred over incineration. However, WTE plants are no complete alternative to landfills, as toxic substances are collected in bottom ashes and air filters which again are stored in landfills (Cucchiella, D'Adamo, & Gastaldi, 2017).

As the waste hierarchy suggests disposal of waste and recovery should only be done, once products can no longer be reused or recycled (Figure 2) (European Parliament & European Council, 2008).

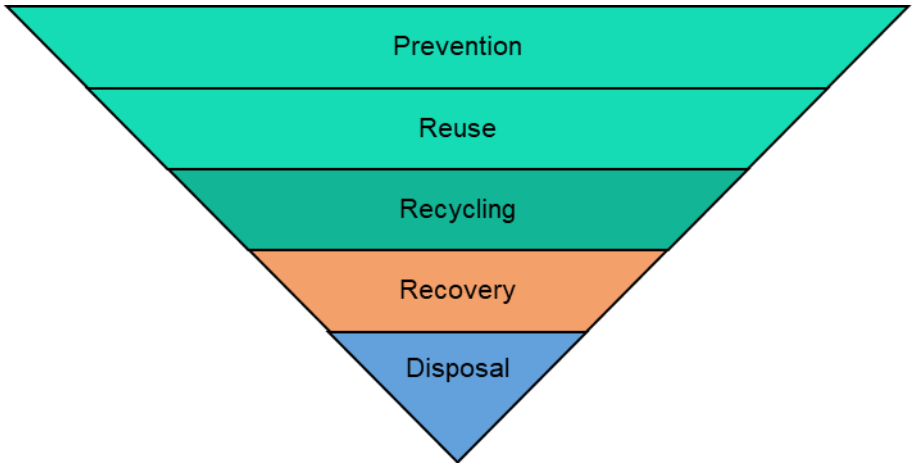

Figure 2: Diagram of the waste hierarchy pyramid showing most favoured waste treatments on top (prevention) and least favoured options at the bottom (disposal).

The circular material rate, a measure indicating how much material is recovered and reused in economy, steadily increased from 11 % in 2010 to 11.7 % in 2016. In textile industry specifically the rate of textile waste being reused in the production of new textiles lies beneath 1 % (MacArthur, 2017; Statistical Office of the European Communities, 2019a).

Aiming to increase these rates, in 2015 the European Commission adopted the Circular Economy Package, a 54 task long action plan aiming to reduce waste and lead the EU into a more sustainable era. By 2018 most of the 54 actions were already completed and a follow-up to the Circular Economy Package was adopted, including more ambitious targets and a revision of the Waste Framework Directive. These changes ensure, that among other things, by the end of 2025 all textiles are collected separately in EU member states (European Commission, 2019).

1.2 Textiles

1.2.1 Cotton

Cotton by far is the most produced natural fibre, and only second to polyester when considering all fibre types. Worldwide, 25.6 million tonnes of cotton were produced in 2013 (Mather & Wardman, 2015).

Cotton consists of 90 % cellulose and therefore is the purest natural form of cellulose. Wood, in comparison, is made of up to 50 % cellulose, depending on the type. The non-cellulosic fraction of cotton contains proteins, pectins, waxes and other substances. The specific composition of cotton varies depending on the species and environmental conditions and

has an influence on the fibre length (“Cotton: Science and technology,” 2007). Higher quality cotton has fibre lengths between 25 and 65 mm, while lower quality fibres have lengths between 10 and 25 mm.

The structure of cotton fibres is built up by multiple layers. Outermost is the wax-coated primary cell wall. Beneath several layers of secondary cell wall spiral around a channel called lumen, which transports a nutritious sap during fibre growth (Mather & Wardman, 2015)

As stated earlier, the main component of cotton is cellulose. Cellulose is a polysaccharide built up from repeating units of cellobiose. Cellobiose itself is a dimer consisting of two β1,4-glycosically bound β-D-glucopyranose units (Figure 3). The degree of polymerisation (DP) represents the number of glucose units aligned in a cellulose chain. Cellulose in the primary cell wall has a degree of polymerisation between 2000 and 6000. The secondary cell wall is made of cellulose of higher molecular weight with a degree of polymerisation of up to 14,000 (“Cotton: Science and technology,” 2007).

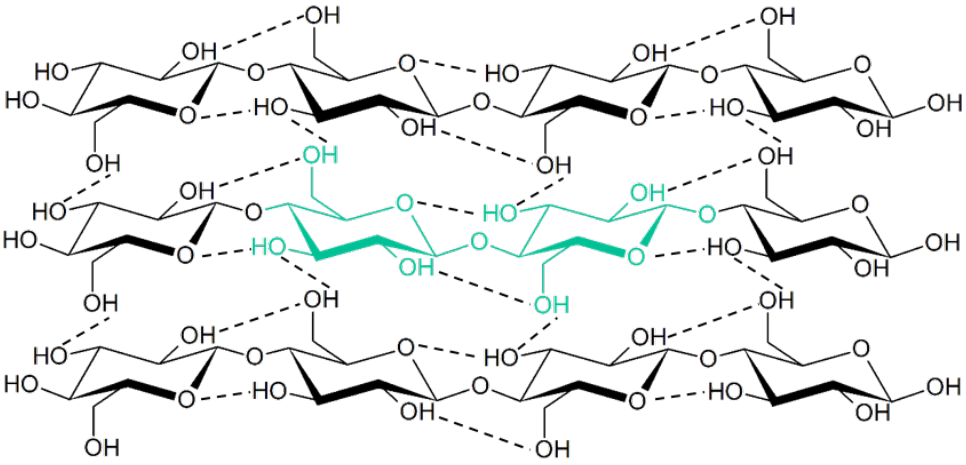

Figure 3: Structure of cellulose. Three chains are shown with inter- and intramolecular hydrogen bonding visualised as dashed lines. One unit of cellobiose is marked in green.

Cellulose strains are directional as they exhibit one reducing end, due to the reactive hemiacetal group, and one non-reducing end. Since each glucose unit contains three hydroxyl groups which can interact with neighbouring oxygen atoms, a tight network of inter- and intra-molecular hydrogen bonds holds the cellulose chains together, forming a strong and rigid crystalline structure. Depending on the orientation of cellulose chains different forms can be distinguished. In cellulose I, the chains are aligned parallel with all reducing ends pointing in the same direction. This form is found in naturally occurring cellulose. Cellulose II, on the other hand, is obtained by dissolving and regenerating cellulose I and occurs in fibres like viscose or Lyocell. In cellulose II chains are oriented with reducing and

non-reducing ends alternating, which is called anti-parallel (Mather & Wardman, 2015). Crystalline regions or varying length are intercepted by more loosely arranged ,or amorphous, regions which are more susceptible to reactions than the tight crystalline structures (Mather & Wardman, 2015).

Cellulose is easily degraded by oxidising agents like hypochlorides, peroxides and acids like sulphuric acid while being recalcitrant towards alkaline treatment ("Cotton: Science and technology," 2007).

1.2.2 PET

Polyethylene terephthalate (PET) is the most abundant textile fibre with over 46 million tonnes produced worldwide in 2013 (Mather & Wardman, 2015).

PET, whose structure can be seen in Figure 4, is produced in a catalytic process from terephthalic acid and ethylene glycol (Gubbels et al., 2018).

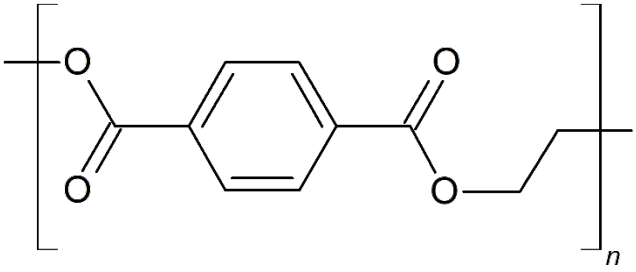

Figure 4: Structure of the repeating unit of PET.

PET fibres used in textiles are up to 50 % crystalline, depending on the application and processing technique. During drawing and spinning PET chains are brought closer together. This results in a stiffer, more crystalline polymer which is suitable for technical textiles with higher mechanical demands (Mather & Wardman, 2015; Sattler & Schweizer, 2011).

1.2.3 Blends

Many of today's textiles are no longer composed of one material but are blends of different fibres like cotton and polyester. In combining multiple materials, the properties of the fabric can be fine-tuned. Cotton is soft and brings a high water absorbency to the fabric, strong PET fibres make fabrics more wrinkle resistant, contribute to textiles durability and lower the cost, due to their cheaper production. The combination of those properties makes polycotton the most common combination of synthetic and natural fibres (Peterson, Palme, Brelid, de la Motte, & Theliander, 2017). It is widely used in service textiles like bed sheet

and towels where the lower moisture absorbency of polyester saves cost and energy in drying of garments (Kalliala & Nousiainen, 1999).

But while textile blends have advantageous properties compared to pure materials, their end-of-life poses a new challenge. The mixed composition of most fabrics does not allow simple reprocessing as it would be able with pure fibres, therefore separation techniques are needed in order to recover the individual materials (Quartinello et al., 2018).

1.3 Recycling methodology

When textile waste is collected separately it undergoes multiple sorting steps. While some pieces are sold in local second-hand stores the largest volume, with up to 48 %, is exported to developing countries for reuse. Textiles which are no longer wearable are often cut into cleaning wipers for industrial use (Hawley, 2014).

As separation of textile blends is still not easily achieved, most of the time such fabrics are shredded and repurposed as insulation material (Payne, 2015). Other recycling options involve chemical, thermal or biological treatments (Hawley, 2014).

The Swedish Research Institute investigated the chemical dissolution of polyester and recovery of its building blocks, while maintaining the cotton fraction (Motte & Palme, 2018). Cotton fibres could then be mechanically recycled. Due to mechanical stress like extensive washing and usage or because of mechanical treatment like shredding and cutting, cotton fibres become shorter which makes subsequent spinning less efficient and results in weaker and more uneven yarns. Processing of short fibres also increases processing wastes (“Cotton: Science and technology,” 2007).

Alternatively, short cotton fibres could be chemically recycled into regenerated cellulose fibres like viscose or Lyocell (“Cotton: Science and technology,” 2007).

Recycling of polyester fibres reduces the amounts of fossil fuels needed and the amount waste incinerated and landfilled. Consequently, the use of non-renewable energy could be reduced by 40-85 % (Shen, 2011). In order to isolate and recycle PET from polyester-cotton wastes, cotton fibres could be degraded by acids or solvents like ionic liquids (De Silva, Wang, & Byrne, 2014; Ouchi, Toida, Kumaresan, Ando, & Kato, 2010). On the other hand, considering environmental and safety factors a biochemical separation process is preferred using highly potent cellulose mixtures combined with optional pre-treatment techniques.

1.4 Cellulases

Cellulases are enzymes capable of hydrolysing β-1,4-glycosidic bonds. Three major types of cellulases are necessary for the fully hydrolysis of cellulose to glucose:

I. Endoglucanases (EC 3.2.1.4),

II. Exoglucanases like exo-1,4-beta-glucosidases (EC 3.2.1.74) and cellobiohydrolases (EC 3.2.1.91)

III. β-glucosidases or cellobiases (EC 3.2.1.21) (Lynd, Weimer, Zyl, & Pretorius, 2002)

In general microorganisms produce multiple types of enzymes from each of the cellulase classes. *T. reesei*, for instance, is a filamentous fungus, known for more than 50 years for its capability to degrade cellulose (Seidl, Seibel, Kubicek, & Schmoll, 2009), that produces two exoglucanases, five endoglucanases and two cellobiases (Vinzant et al., 2001).

Endoglucanases are responsible for rapidly decreasing cellulose's degree of polymerisation by randomly cutting cellulose in amorphous regions, creating new chain ends (Kleman-Leyer, Agosin, Conner, & Kirk, 1992). The active site of endoglucanase 1 (EG1) and EG3 is a groove formed by loops in the enzyme's tertiary structure which is clearly visible in (Figure 5) (Henriksson, Ståhlberg, Isaksson, & Pettersson, 1996; Sandgren et al., 2001). The generation of new chain ends results in a synergism with exoglucanases.

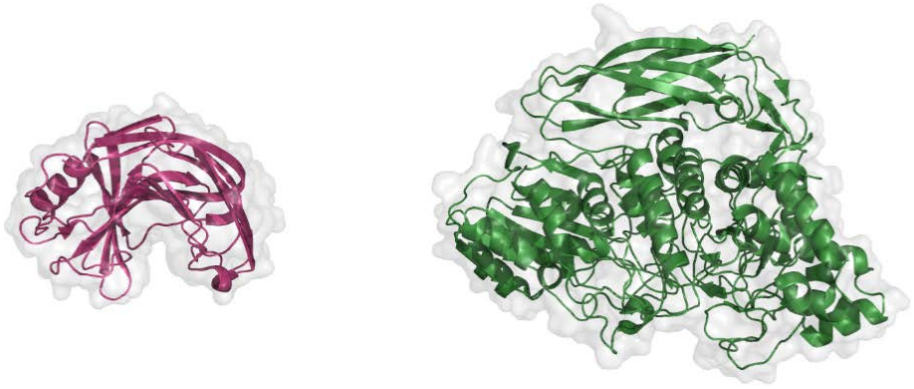

Figure 5: Endoglucanase 3 (pink) and β-glucosidase 1 (green) from *T. reesei*. The groove of the active site is clearly visible. Structures were obtained from protein data base EG3: 1H8V; β glucosidase: 3ZYZ. Rendered in PyMOL

Exo-1,4-beta-glucosidases and cellobiohydrolases start the degradation of cellulose specifically at reducing or non-reducing chain ends, releasing glucose and cellobiose respectively (Lynd et al., 2002). In Exoglucanases the active site in shape of a tunnel is

formed by four loops in the case of cellobiohydrolases 1 (CBH1) and two loops in CBH2 (Divne et al., 1994). Once CBH1 and CBH2 attached to cellulose chain ends they consecutively cleave multiple bonds before the enzyme dissociates (Imai, Boisset, Samejima, Igarashi, & Sugiyama, 1998).

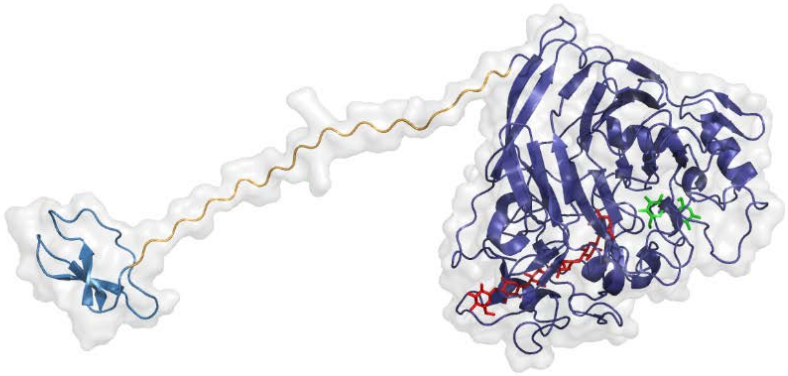

Figure 6: Exoglucanase 1 from *T. reesei*. Catalytic domain (dark blue) and cellulose binding module (light blue) are connected through a flexible linker (yellow). A short cellulose chain (red) and the catalytic product cellobiose (green) are visible in the active site. Enzyme was assembled in Chimera and rendered in PyMOL with structures for the catalytic domain (7CEL) and CBM (1CBH) obtained from protein data base. Sequence of the linker was obtained from: https://www.ncbi.nlm.nih.gov/protein/P62694.1

Most endo- and exoglucanases have cellulose binging modules (CBM) which help adhere to the polysaccharide. Commonly CBMs are attached to the catalytic domain through a flexible linker (Figure 6). While CBH1 has just one and EG3 lacks a CBM (see Figure 5) other enzymes contain multiple binding modules (Lynd et al., 2002). The adherence of CBMs to cellulose is thought to be driven by van der Waals forces and interaction of polarised aromatic amino acids with the pyranose rings of glucose (Lehtiö et al., 2003).

The degradation of cellulose is finalised in another enzymatic cooperation as cellobiose, released through cellobiohydrolases, is hydrolysed to glucose by β-glucosidase (Lynd et al., 2002).

A schematic visualisation of the degradation of cellulose by the various cellulase enzymes can be seen in Figure 7.

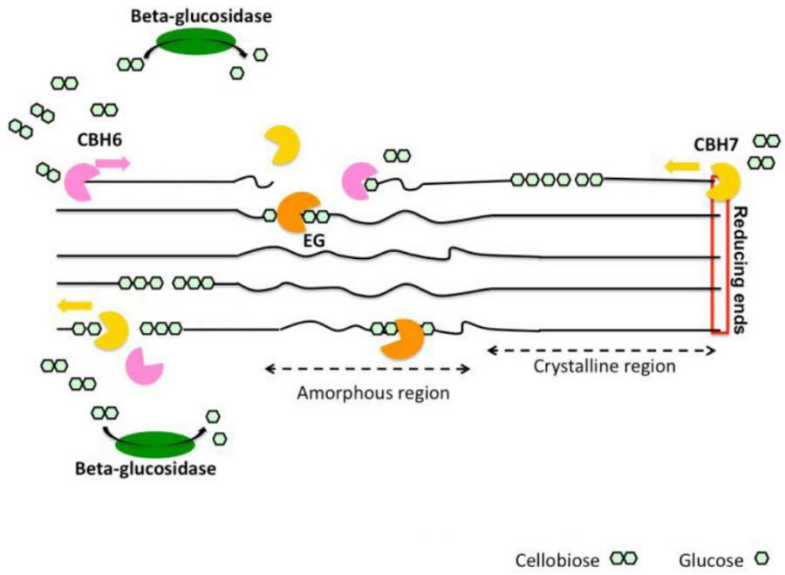

Figure 7: Illustration of synergetic hydrolysis of cellulose by different cellulases. Exoglucanases (yellow and pink) start the degradation at cellulose chains ends. Endoglucanase (orange) cut chains in amorphous regions and B-glucosidase releases glucose from cellobiose. Picture adapted from Dimarogona et al. (Dimarogona, Topakas, & Christakopoulos, 2012)**.**

1.5 Improving cellulose degradation

As previously described, cellulose is arranged in a tight network, which makes it hard for enzymes to attach. Even though multiple enzymatic synergies are in place, the biochemical degradation of cellulose is still a slow process, achieving up to 80 % conversion within 120 hours (Adewuyi & Deshmane, 2015).

Therefore, effective pre-treatments or a combination of multiple pre-treatment methods could facilitate the enzymatic degradation by making cellulose more accessible to the enzymes (Zheng, Pan, & Zhang, 2009).

1.5.1 Mechanical pre-treatment

During mechanical treatment like cutting and milling textile fibres decrease in length and the overall surface area increases. As exoglucanases can only degrade cellulose at chain ends, a decrease in the degree of polymerisation, resulting in shorter fibres and more chain ends promotes exoglucanase activity. Increasing the surface area improves the accessibility of cellulose for endoglucanases which adhere to the surface of fibres (Lee & Fan, 1982).

Smaller particles also facilitate mass transfer and efficient mixing during hydrolysis, ensuring that enzymes come in contact with the polysaccharide.

1.5.2 Ultrasonic treatment

Ultrasound are acoustic waves with a frequency from 10 kHz to 20 MHz. During treatment with ultrasonic waves small bubbles spawn in the liquid medium. These bubbles burst within several nanoseconds releasing a shock wave of energy which results in high pressures of 50 MPa and localised temperature hotspots of up to 5000 °C. Unsymmetrical collapse of bubbles can additionally create directional microjets with high speeds in the range of 100 m/s. This effect is called cavitation and is the main mechanism of ultrasonic treatment (Luo, Fang, & Smith, 2014).

These strong forces induced via cavitation can fractionate the rigid cellulose chains and loosen the polysaccharide matrix, creating more amorphous regions for enzymes to attack (Adewuyi & Deshmane, 2015; Nguyen & Le, 2013).

As such bursts would also lead to changes in the enzyme structure (Nguyen & Le, 2013) high intensity ultrasound is preferred as a treatment prior enzyme addition. But ultrasound is also reported to promote mass transfer and thereby accelerate reaction rates (Luo et al., 2014). While high intensity ultrasound was reported to damage enzymes, low intensity ultrasound enhances enzyme activity (Sulaiman, Ajit, & Chisti, 2013). Ultrasonic waves in the range of 20 to 50 kHz are reported of being beneficial during enzymatic treatments (Luo et al., 2014). In some cases, ultrasound decreased the cellulase activity while still yielding positive results during the hydrolysis of cellulose. This implies that the desired effects ultrasonic waves have on the structure of cellulose overcome the undesired decrease in enzyme activity (Szabó & Csiszár, 2013).

Applying the right frequency during ultrasonic treatment is important. While cavitational forces are, in theory, weaker when applying higher frequencies, because bubbles grow smaller and collapse sooner than at lower frequencies, the energy release of single bubbles was reported to be higher at high frequencies. Additionally, higher temperatures (4730-5930 °C) were measured in cavitation bubbles induced by high frequency ultrasound than in bubbles created by low frequency ultrasound (3430-3930 °C). The (Luo et al., 2014). During ultrasound aided cellulose hydrolysis glucose yields increased when the frequency was raised from 20 to 611 kHz. But when frequencies were increased even further, lower glucose yields were observed. After further experiments with ultrasonic frequency and power, the authors proposed, that there might be an optimal ultrasonic power for each

frequency, since different combinations of these two parameters yielded similar results (Adewuyi & Deshmane, 2015).

Ultrasonic treatment appears to be a complex topic. Choosing the right parameters for a given process is crucial for a successful treatment. Applying too high intensities can damage enzymes or equipment, while at too low intensities no effect might be observed. At the same time specific parameters cannot be proposed, since results vary considerably with the material and enzymes used (Luo et al., 2014).

1.5.3 Alkaline pre-treatment

As previously mentioned, cellulose is readily degraded in acid solutions but rather resilient towards alkaline conditions. Still some effects of sodium hydroxide solutions on cellulose have been reported. At high temperatures alkaline solutions can remove glucose molecules from reducing ends at a very slow rate. The main effect of alkaline solutions, though, is swelling of cellulose fibres (Mather & Wardman, 2015).

This effect was first discovered by John Mercer in 1850, who found that upon treatment with concentrated sodium hydroxide solutions cellulose fibres would swell in diameter and shrink in length. Due to this change the dye intake would increase significantly (Mather & Wardman, 2015). During this process, since called mercerisation, alkaline solutions first penetrate amorphous regions, causing swelling in those areas before crystalline regions of cellulose are affected. Mercerisation is performed at elevated temperatures and high NaOH concentrations (Budtova & Navard, 2015).

Since polyester is very susceptible to alkaline hydrolysis, particularly at hot temperatures (Kosmidis, Achilias, & Karayannidis, 2001), a heated NaOH-treatment is an unfavourable process for polycotton when the goal is to preserve PET fibres.

Alkaline pre-treatment might still be a viable option for this goal since cold NaOH solutions have also been reported to dissolve cotton fibres. While NaOH is often combined with other chemicals like urea for a faster dissolution, up to 80 % solubility of cellulose were observed in NaOH at -5 °C without the addition of any other chemicals. Additionally, the combination of steam-exploded cellulose with alkaline treatment was found particularly effective (Budtova & Navard, 2015).

1.5.4 Steam explosion

Steam explosion is a physical pre-treatment in which biomass is subjected to pressurised water steam for a given time followed by a sudden decompression. Chemicals can be added

to the water if certain reactions are anticipated (Alvira, Tomás-Pejó, Ballesteros, & Negro, 2010).

Steam explosion is a common pre-treatment for lignocellulosic material, as hemicelluloses can be extracted during the incubation time at high temperatures. But steam explosion can also be a beneficial pre-treatment for cellulose due to the mechanical forces arising during the decompression phase. The rapid expansion of water defibrillates fibres, creating physical space inside the complex cellulose network (Schultz, Blermann, & Mcginnis, 1983). This could increase accessibility for enzymes, hence improving the hydrolysis.

1.5.5 Solvent treatment

Dissolution of cellulose in solvents is also a widely discussed topic. N-Methylmorpholine N-oxide (NMMO) has proven effective and is also used in the Lyocell process (Fink, Weigel, Purz, & Ganster, 2001; Mather & Wardman, 2015). Apart from strong hydrogen bonding cellulose's recalcitrance is thought to come from hydrophilic interactions (Lindman, Karlström, & Stigsson, 2010). Since Ionic liquid solvents (IL) often exhibit amphiphilic properties they have recently become popular solvents for the dissolution of cellulose (Jeihanipour, Karimi, Niklasson, & Taherzadeh, 2010; Swatloski, Spear, Holbrey, & Rogers, 2002). They are used in combination with cosolvents like DMSO of DMF to further improve the dissolution process (Zhao, Liu, Wang, & Zhang, 2013). DMSO and DMF, both aprotic dipolar solvents, have also been reported to interact with cellulose on their own. Although they do not dissolve cellulose like ionic liquids, they were able to create a dispersion of cellulose nanocrystals (Viet, Beck-Candanedo, & Gray, 2007).

Dihydrolevoglucosenone (Cyrene, Figure 8) is a rather new addition to the solvent market and poses an interesting possibility as treatment for cellulose. Unlike most solvents and ionic liquids, it is bio-based, derived from cellulose in a two-step reaction, and non-toxic. Like DMSO and DMF Cyrene is a dipolar, aprotic solvent (Sherwood et al., 2014) and therefore could interact with cellulose chains. It is thought to swell cellulose similar to sodium hydroxide improving the accessibility to enzymes during the hydrolysis.

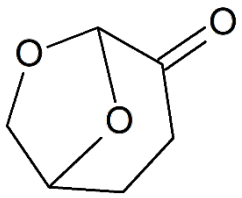

Figure 8: Structure of Dihydrolevoglucosenone (Cyrene)

1.5.6 Continuous removal of hydrolysis products via ultrafiltration

It has been reported various times that enzymes are inhibited by their products (Frieden & Walter, 1963; Xiao, Zhang, Gregg, & Saddler, 2004). Cellobiase in *T. reesei* is very prone to glucose inhibition and exoglucanases are sensitive to inhibition by cellobiose (Lynd et al., 2002).

Therefore, by continuously removing these hydrolysis products from the process, high cellulase activities could be maintained leading to a faster and more complete hydrolysis (Andrić, Meyer, Jensen, & Dam-Johansen, 2010b).

Figure 9 shows the process flow diagram of a reactor with continuous product removal as it was used during this thesis.

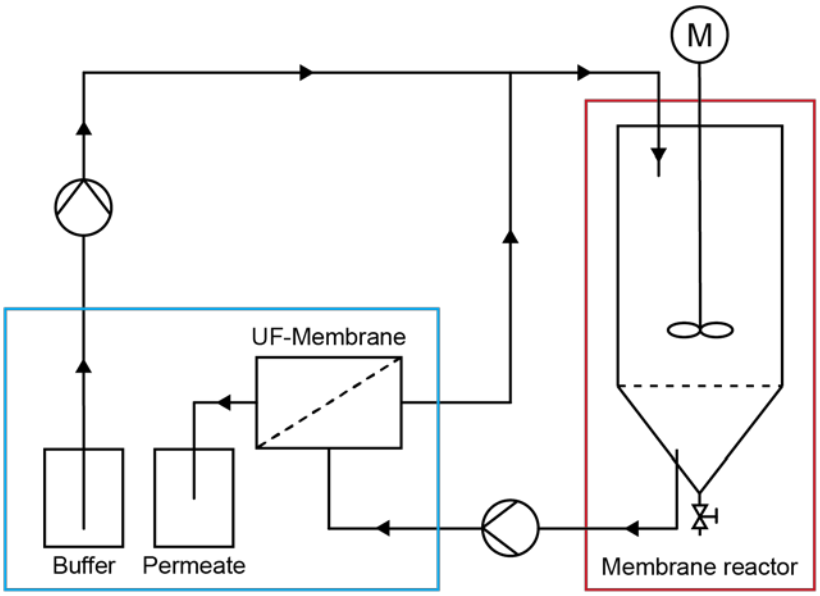

Figure 9: Process scheme of the membrane-reactor. The reactor is placed inside a heated chamber (red), buffer, permeate and ultrafiltration membrane are cooled on ice (blue).

1.6 Value-added products

During the hydrolysis of cotton a high quantity of glucose is generated, which can be used for the production of value-added products.

2,5-Furandicarboxylic acid (FDCA, Figure 10) is a substance that has recently attracted attention in the field of polymer sciences. It can be used as a substitute for terephthalic acid in the production of polyesters. Upon polymerisation of FDCA with ethylene glycol (EG) polyethylene furanoate (PEF), a chemical analogon to PET, is produced. While having similar properties, PEF is thought to replace PET due to its environmental benefits. Not only

is the polymerisation process of PEF more energy efficient, but the building blocks, FDCA and EG, can be derived from biological sources (Eerhart, Faaij, & Patel, 2012). Furandicarboxylic acid is either synthesised by oxidative dehydration of glucose (Pellis, Herrero Acero, Gardossi, Ferrario, & Guebitz, 2016) or by conversion of 5-hydroxymethylfurfural (HMF), which can also derived from glucose (Rosatella, Simeonov, Frade, & Afonso, 2011).

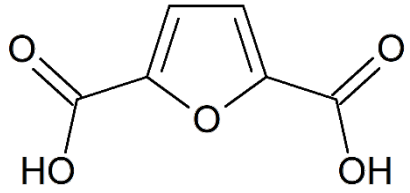

Figure 10: Structure of 2,5-Furandicarboxylic acid (FDCA)

Glucose is the most important energy source for all organisms (Berg, Tymoczko, & Stryer, 2013). Therefore, glucose can be used to generate biomass as product or use microorganisms for the conversion of glucose to other valuable substances.

The production of ethanol by *S. cerevisiae* is a simple process that is historically well known for its use in wine making and brewing. Alongside its use in the food industry ethanol is regarded as one as the best liquid fuel alternatives to fossil fuels. Most of the ethanol is produced from sugars from corn or sugarcane in the USA and Brazil. Due to conflicts of interest during the production of chemicals from food-plants, the development of processes which use lignocellulosic material as substrate is investigated (Lin & Tanaka, 2006).

Glycolysis, the metabolic pathway that breaks down glucose into pyruvate, is followed by alcoholic fermentation, a two-step process during which pyruvate is converted to ethanol and the reduced cofactor (NADH) from glycolysis is regenerated. Enzymes involved in alcoholic fermentation are pyruvate decarboxylase and alcohol dehydrogenase. The overall reaction is summarised by equation (1).

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2 \qquad (1)$$

Usually ethanol fermentation is regarded an anaerobic process. Most organisms prefer respiration over fermentative processes, as respiration yields more energy. Nonetheless, in *S. cerevisiae* fermentation is, even under aerobic conditions, the predominant metabolic pathway. This phenomenon, called Crabtree effect, is thought to be due to glucose inactivating respiration enzymes or repressing their synthesis (G. M. Walker, 1998).

Metabolic engineering gave rise to an *E. coli* strain which is capable of converting glucose to 1,4-butandiol, a chemical predominantly derived from fossil fuels. As 1,4-butandiol is

used for the production of over 2.5 million tons of polymers per year the biochemical production of this building block from glucose would substantially reduce the environmental impact (Yim et al., 2011).

Glucose from cotton hydrolysis and other waste streams is a versatile resource which can be upcycled to various products of higher value as demonstrated in these examples. Not only can this drastically reduce the use of fossil fuels, but it is another important step into a future of complete circular economy.

1.7 Objectives

Increased awareness and legislative changes gave rise to Tex2Mat, an Austrian joint project of several universities and partners within the textile- and material industry. The project's aim is to develop a recycling process for multi-material textile wastes suitable for small and medium sized enterprises (ecoplus. Niederösterreichs Wirtschaftsagentur GmbH, 2017)

This master thesis was part of the Tex2Mat project, with the specific goal to develop an enzyme aided process for the recycling of polyethylene terephthalate from cotton/PET blended textiles. As most of today's PET is produced from limited fossil resources which release greenhouse gas upon combustion, an efficient recycling process leading to a closed-loop system for polyester textiles is needed. This way valuable resources could be reused multiple times before they are eventually replaced with bio-based alternatives (Shen, 2011).

2 Material

2.1 Textile samples

Different samples were provided by Salesianer, a project partner. These were end-of-life rental textiles, which were originally composed of 50 % cotton and 50 % PET. After extensive machine-washing the cotton content decreased to approximately 45 %. Experiments were mainly conducted with these fibres, which were supplied in different particle sizes.

Denim were cotton fibres derived from indigo-dyed textile. Denim textiles were dissected by hand to fibres of approximately 4 mm length.

Bed sheet samples were composed of 50 % cotton and 50 % PET. Like denim samples these textiles were cut by hand.

Pure cotton and PET were supplied with a particle size of 0.5 mm by project partners and were used for comparison with mixed textiles.

Pictures of all textile samples used in the experimental section can be seen in (Figure 11).

Figure 11: Textile samples used during experiments.

2.2 Equipment

Infinite M200 PRO plate reader	Tecan, Switzerland
HPLC 1260 Infinity II	Agilent, USA
ICSep Ion-300 column	Transgenomic, USA
HPLC 1290 Infinite II	Agilent, USA
Poroshell 120 ec-c18 column	Agilent, USA
Centrifuge 5427 R, Rotor: FA-45-48-11	Eppendorf, Germany
Centrifuge J2-MI, Rotor: JA-10	Beckman Coulter, USA
Spectrum 100 FT-IR spectrometer	Perkin Elmer, USA
Ismatec Reglo ICC, 4 channel, 8 roller pump	Cole-Parmer, USA
Temperature controller LTR 2500S	Juchheim Solingen, Germany
pH electrode SevenCompact	Mettler Toledo, USA
Mini-PROTEAN Tetra electrophoresis cell	Bio-Rad, USA
PowerPac Universal Power Supply	Bio-Rad, USA
ChemiDoc MP gel imaging system	Bio-Rad, USA
Digital Sonifier 250	Branson Ultrasonics, USA
Proclean 3010	Ulsonix, Germany
Thermomixer comfort	Eppendorf, Germany
Pipettes	Gilson, France

2.3 Chemicals

3,5-Dinitrosalicylic acid	Sigma-Aldrich, USA
Acetic acid ≥ 99 %	Sigma-Aldrich, USA
$C_4H_4O_6KNa \cdot 4H_2O$	Sigma-Aldrich, USA
Citric acid	Carl Roth, Germany
Coomassie Blue G 250	VWR, USA
Cyrene™	Sigma-Aldrich, USA
D-glucose	Sigma-Aldrich, USA

Dimethyl sulfoxide (DMSO)	Sigma-Aldrich, USA
Ethanol absolute	VWR, USA
K_2HPO_4	Sigma-Aldrich, USA
$K_4[Fe(CN)_6] \cdot 3H_20$	Carl Roth, Germany
KH_2PO_4	Sigma-Aldrich, USA
Na_2CO_3	Sigma-Aldrich, USA
NaOH	Sigma-Aldrich, USA
Peptone	Carl Roth, Germany
Protein standard	Sigma-Aldrich, USA
TGS Buffer (10x)	Bio-Rad, USA
Triton X-100	Sigma-Aldrich, USA
Yeast extract	Becton Dicinson & Co., USA
$ZnSO4 \cdot 7H_2O$	Sigma-Aldrich, USA

2.4 Kits and other material

Protein assay kit	Bio-Rad, USA
4-15% Mini-PROTEAN TGX Stain-Free gel	Bio-Rad, USA
Protein Marker IV, peqGOLD	VWR, USA
Filter paper grade 1, 75 x 100 mm	Whatman, UK
Filter paper grade 595 1/2, d = 150 mm	Whatman, UK

2.5 Buffers and solutions

Citric buffer 20 mM, pH 4.8

Carrez reagent C1:	5.325 g $K_4[Fe(CN)_6] \cdot 3H_20$ 50 ml Milli-Q-H_2O
Carrez reagent C2:	14.400 g $ZnSO_4$ 50 ml Milli-Q-H_2O
Glucose stock for activity assay:	20 mM D-glucose Milli-Q- H_2O

Sample washing:	Triton X100 (5 g/l) Na_2CO_3 (100 mM) Milli-Q- H_2O
DNS solution:	47.84 mM Dinitrosalicylic acid 0.4 M NaOH 1.06 M $C_4H_4O_6KNa \cdot 4H_2O$ (Rochelle salt) Milli-Q- H_2O
YPD medium:	1 % w/v yeast extract 1 % w/v peptone 2 % w/v D-glucose Milli-Q- H_2O
K-phosphate buffer 0.1 mM, pH 7.0:	390 ml 0.2 M KH_2PO_4 910 ml 0.2 M K_2HPO_4.
0.2 M KH_2PO_4:	27.2 g KH_2PO_4 diluted in H_2O and adjusted to 1 litre.
0.2 M K_2HPO_4:	34.8 g K_2HPO_4 diluted in H_2O and adjusted to 1 litre.
SDS PAGE de-staining solution:	60 % MQ-H_2O 30 % Ethanol 10 % Acetic acid
SDS PAGE staining solution:	De-staining solution 0.125 % Coomassie Blue
SDS PAGE running buffer:	10 % TGS Buffer 90 % Milli-Q- H_2O

2.6 Organisms and enzymes

Cellic® CTec3 cellulase	Novozymes, Denmark
NS®-52143 cellulase	Novozymes, Denmark
Cutinase (Novozym® 51032)	Novozymes, Denmark
Saccharomyces cerevisiae	DSMZ, Germany
Chlorella sorokiniana	DSMZ, Germany
Trichoderma reesei	DSMZ, Germany

3 Methods

3.1 Enzyme characterisation

Different cellulase mixtures were obtained from Novozymes and compared in multiple regards. Composition was studied via biochemical characterization which include SDS PAGE and activity assays as well as protein concentration. Furthermore, activity measurements were conducted in the course of various stability tests for cellulases and cutinase.

3.1.1 SDS PAGE

Enzymes were diluted to approximately 1.5 mg/ml in citric buffer. 20 µl enzyme dilution were mixed with the same amount 2x Laemmli sample buffer and incubated for 5 minutes at 99 °C while shaking. 10 µl enzyme solution and 5 µl protein marker IV (peqGOLD) were loaded in respective wells of 4-15 % Mini-PROTEAN™ TGX Stain-Free™ Protein gels (Bio-Rad, USA).

Gels were run at 100 V for 45 minutes. Afterwards, gels were stained in staining solution for 45 minutes and subsequently de-stained for two hours in de-staining solution. After 45 minutes the de-staining solution was replaced with fresh one.

3.1.2 Protein concentration assay

Protein concentrations were determined with Bio-Rad protein assay, based on the Bradford method.

A calibration was performed with BSA standards (Protein standard, Sigma Aldrich) of the following concentrations: 2 mg/ml, 1 mg/ml, 0.5 mg/ml, 0.25 mg/ml, 0.2 mg/ml, 0.125 mg/ml, 0.1 mg/ml, 0.0627 mg/ml, 0.05 mg/ml, 0.03125 mg/ml and 0.025 mg/ml.

10 µl standard or enzyme dilution were pipetted into a 96 well plate and 200 µl of 1:5 diluted Bio-Rad Protein Assay Dye Reagent Concentrate were added to each well. After 5 minutes of incubation at 400 rpm and room temperature, absorption was measured at 595 nm.

3.1.3 Filter paper assay (total cellulase activity assay)

The determination of reducing sugars with 3,5-dinitrosalicylic acid is based on a redox reaction of the sugar's aldehyde group with DNS. The aldehyde group is oxidised, and DNS

is reduced to 3-amino-5-nitrosalicylic acid, whose red colour can be measured photometrically.

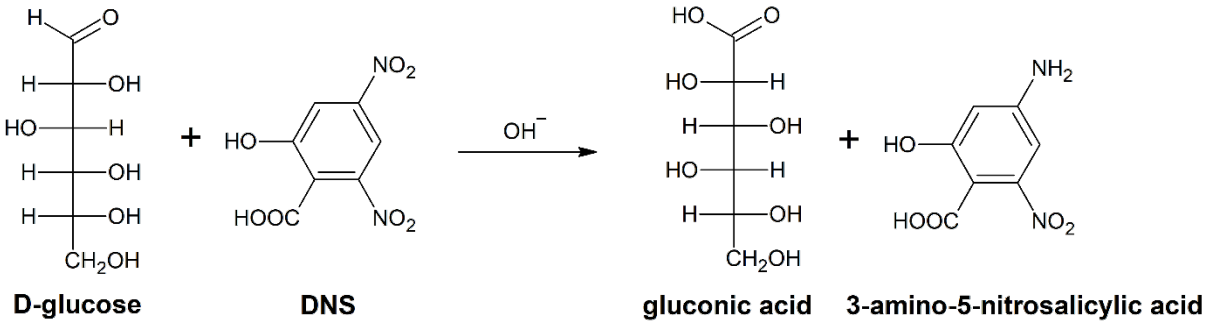

Figure 12: Underlying reaction of the cellulase activity assay with DNS

Each freshly prepared DNS solution was calibrated. Therefore 200 µl of each glucose standard, ranging from 0 to 20 mM, were mixed with 200 µl NaOH (1M) and 200 µl DNS solution before they were boiled for 5 minutes in a water bath. Standards were then diluted with 400 µl MQ-H_2O and 200 µl of each standard transferred into wells of a 96 well plate. The absorbance at 540 nm was measured on the plate reader. Each standard was prepared in triplicates.

For the activity assay a rolled filter paper strip (Whatman grade 1, ca. 50 g) was placed in 1 ml citric buffer and equilibrated to 50 °C. A defined volume of 100 µl of cellulase cocktail (diluted 1:1000) were added and the reaction was stopped after different time points (0, 5, 10, 20, 40, 60 minutes) by addition of 500 µl 1 M NaOH. For time point 0, NaOH was added before the enzyme solution.

500 µl DNS solution were added and the samples were boiled in a water bath for 5 minutes. Afterwards the samples were diluted with 1 ml MQ-H_2O. 200 µl of each dilution were transferred into a 96 well plate and the absorbance was measured at 540 nm on the plate reader.

3.1.4 Cellulase stability in Cyrene solution

To observe the effects of Cyrene on enzyme activity cellulases were incubated at hydrolysis conditions with addition of Cyrene. Therefore 1 ml solutions containing 1 % cellulase cocktail and 1 % respectively 2.5 % Cyrene in citric buffer were incubated in micro-reaction tubes at 50 °C, 300 °C on a thermomixer.

At defined time points a tube was retrieved and stored at -20 °C until the activity assay was performed. Before measuring the activity, samples were diluted 10-fold to reach the desired

dilution for the assay. Blanks containing only 1 % enzyme in citric buffer were also measured for comparison.

For this assay a new calibration was performed, taking the addition of Cyrene into account (Table 1).

Table 1: Pipetting scheme for the preparation of Cyrene-containing glucose standards for the cellulase activity assay.

Glucose [mM]	Stock [µl]	buffer	buffer + 1 % Cyrene [µl]	buffer + 2.5 % Cyrene [µl]
20	1000	0	0 + 10	0 + 25
18	900	100	90 + 10	75 + 25
16	800	200	190 + 10	175 + 25
14	700	300	290 + 10	275 + 25
12	600	400	390 + 10	375 + 25
10	500	500	490 + 10	475 + 25
8	400	600	590 + 10	575 + 25
6	300	700	690 + 10	675 + 25
4	200	800	790 + 10	775 + 25
2	100	900	890 + 10	875 + 25
1	50	950	940 + 10	925 + 25
0	0	1000	990 + 10	975 + 25

3.1.5 Influence of ultrasound on cellulase activity

20 µl enzyme were diluted in 1980 µl citric buffer and incubated for 7 days at 50 °C, 300 rpm. Enzymes were exposed to ultrasound for 20 minutes at 42 kHz, 300 W sonic power and 50 °C (Ulsonix Proclean 3010) 3 and 6 hours after incubation start. The treatment was repeated at the same time for 5 days. Samples were taken daily, diluted 1:10 and used for the activity assay as described above.

3.1.6 Cutinase activity assay

This assay is based on the hydrolysis of the substrate para-nitrophenyl butyrate to *p*-nitrophenol which can be quantified photometrically.

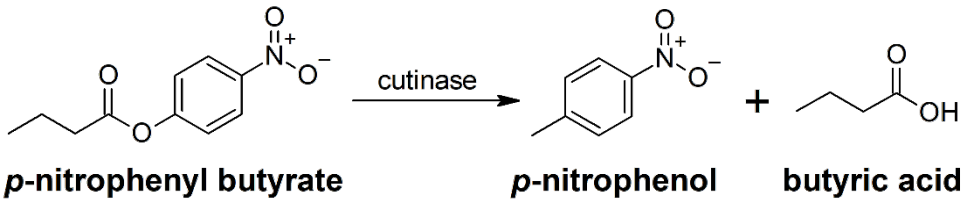

Figure 13: Enzymatic hydrolysis of *p*-nitrophenyl butyrate to *p*-nitrophenol during the cutinase activity assay.

9.2 µl (11 mg) p-nitrophenyl butyrate (pNPB) were diluted in 1 ml DSMO, which corresponds to a concentration of 52.1 mM. The solution was further diluted with K-phosphate buffer to a PNPB concentration of 0.116 mM. 20 µl cutinase, diluted to a linear reaction rate with K-phosphate buffer, were brought to reaction with 200 µl PNPB solution in a 96 well plate and immediately measured in constant intervals for 5 minutes at 405 nm on the plate reader. Each sample was measured in triplicates. A blank, containing only buffer instead of enzyme, was subtracted from enzyme absorptions.

The activity in U/ml was calculated using the following equation:

$$\text{Activity} = \frac{\text{rate} \cdot V_t \cdot d_f}{\varepsilon \cdot d \cdot V_\text{e}} \qquad (2)$$

Rate = Δ Abs/ Δ time

ε = molar extinction coefficient = 8.5 M^{-1} cm^{-1}

V_t = total reaction volume = 220 µl

V_e = enzyme volume = 20 µl

d_f = dilution factor of the enzyme

d = path length = 0.65 cm

The path length was determined experimentally according to a protocol from the biotechnology company Promega (Promega Corporation, 2009).

3.2 Analytical methods

3.2.1 HPLC (High pressure liquid chromatography)

3.2.1.1 Carrez precipitation

960 µl sample were mixed thoroughly with 20 µl of potassium hexacyanoferrate(II) trihydrate (C1 solution). 20 µl zinc sulphate heptahydrate (C2 solution) were added and samples were mixed for 5 minutes. Samples were then centrifuged for 30 min at 17,131 × g, 4 °C. The supernatant was used for HPLC analysis.

3.2.1.2 Glucose, ethanol and Cyrene quantification

Carrez-precipitated samples were filtered through a 0.45 µm polyamide filter into HPLC vials before measurement on the HPLC. The HPLC system used was an Agilent 1260 Infinity II equipped with an ICSep Ion-300 column (Transgenomic) and a refraction index detector. 40 µl sample were injected. H_2SO_4 (0.01 M) was used as the mobile phase, with a flow rate of 0.325 ml/min at 45 °C.

Glucose, ethanol and Cyrene standards were prepared 0.01 to 1 g/l.

3.2.1.3 Methanol precipitation

500 µl sample were mixed with 500 µl ice cold methanol and acidified to pH 3 with 6 N HCl. Samples were then centrifuged for 15 minutes at 17,131 × g, 4 °C. The pellet was discarded while the supernatant was used for further analysis via HPLC.

3.2.1.4 Terephthalic acid quantification

Methanol-precipitated samples were filtered through a 0.45 µm polyamide filter into HPLC vials. The HPLC system used was an Agilent 1290 Infinite II with a C-18 column and a diode array detector. Water, methanol and 10 % formic acid were used as solvents and run at a gradient as shown in Table 2. 10 µl sample were injected and run with a flow rate of 0.85 ml/min at 40 °C column temperature.

Concentrations of terephthalic acid and bis(2-Hydroxyethyl) terephthalate (BHET) standards ranged from 0.001 to 1 mM.

Table 2: Solvent gradient during terephthalic acid quantification via HPLC.

Time [min]	Water [%]	Methanol [%]	Formic acid (10%) [%]
0	85	5	10
1	80	10	10
8	40	50	10
10	0	90	10
20	0	100	0

3.2.2 FTIR analysis

The purity of PET can be determined via FTIR analysis. In the spectrum of cotton a very prominent peak, deriving from O-H stretches, can be seen between 3600 and 3000 cm^{-1}. Since PET does not have any hydroxyl groups this peak is not visible after successful removal of cotton from mixed fibres. At 1020 cm^{-1} a large peak associated to C-O stretches in cellulose is visible. In mixed fibres this peak overlaps with the two peaks derived from C-O-C stretching in aromatic ester bonds (1260 and 1130 cm^{-1}) occurring in PET (Figure 14: FTIR spectra of pure cotton and PET showing all relevant peaks for analysis.). Reduction of these two peaks can be observed easily. A full list of visible peaks is given in Table 3.

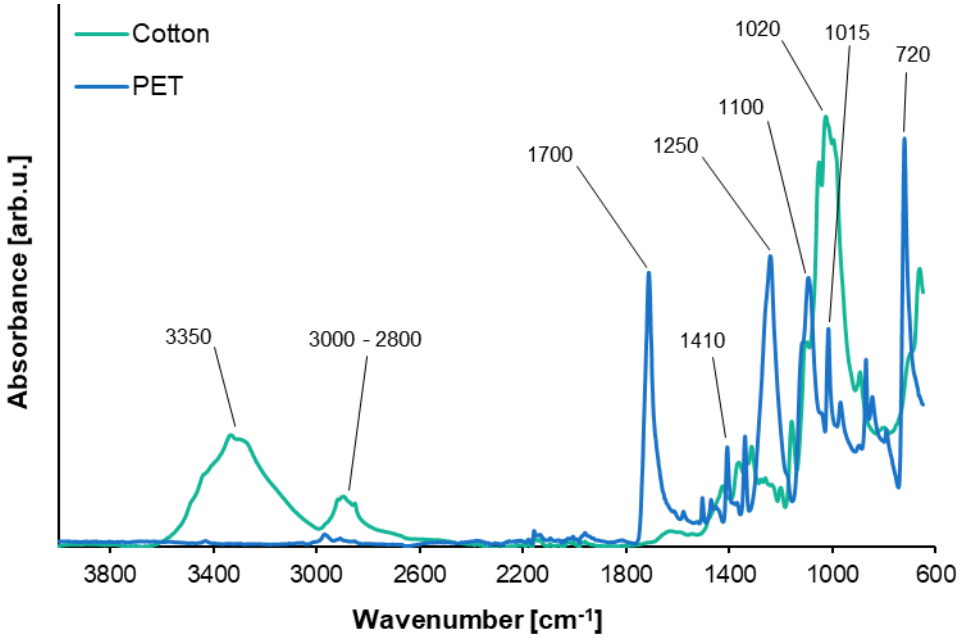

Figure 14: FTIR spectra of pure cotton and PET showing all relevant peaks for analysis.Table 3: List of characteristic IR spectroscopy bands of cotton and PET.

Wavenumber [cm^{-1}]	group	Compound class	fibre
3350	O-H stretch	alcohol	cotton
3000-2800	C-H stretch	alkane	cotton, PET
1700	C=O stretch	ester	PET
1250	C-O-C stretch	aromatic ester	PET
1100 + 1015	C-O stretch	ester	PET
1020	C-O stretch	aliphatic ether	cotton
720		aromatic ring	PET

Dry samples were measured from 4000 to 600 cm^{-1} and 30 accumulations on a Perkin Elmer Spectrum 100 FTIR spectrometer. Spectra were base-line corrected and normalised at 720 cm^{-1}, as the terephthalic acid content of the fibres does not change during cotton hydrolysis.

Pure PET samples were normalised at 1410 cm^{-1} which has been reported to be a reliable reference point for PET (Walls, 1991). The zero-point was set to 1491.5 cm^{-1} allowing a good comparison of the spectra. To determine changes in crystallinity the intensity (height) of peaks at 1120 and 1100 cm^{-1} and the areas of peaks at 1341 and 1410 cm^{-1} are compared. In amorphous PET the ration I_{1120}/I_{1100} is lower than in crystalline PET and both peaks are shifted to lower wavenumbers. The ratio A_{1341}/A_{1410} is also lower in amorphous PET as the peak at 1341 cm^{-1} is lower or even absent. The peak at 1250 cm^{-1} appears as one big peak in crystalline PET, whereas two lower peaks are visible in amorphous PET instead (Donelli et al., 2009).

3.2.3 Weight loss

Determination of weight loss is a simple method to determine the grade of degradation. After experiments solids were washed and dried and their weight was compared to the initial weight.

$$\text{Degradation } [\%] = 1 - \frac{M_0}{M_t} * 100 \qquad (3)$$

M_0 = Initial mass

M_t = Mass after hydrolysis

3.3 Pre-treatment studies

3.3.1 Mechanical pre-treatment

Bed sheet (50 % cotton, 50 % PET) samples were dissected by hand and scissors until single fibres with a maximum length of 5 mm were obtained. Salesianer, cotton and PET samples were provided milled by project partners and cut denim samples were available at the institute.

3.3.2 Washing

All textile samples were washed with Triton X100 (5 g/l), Na_2CO_3 (100 mM) and MQ-H_2O for 30 minutes at 50 °C each. Afterwards they were rinsed with purified water until the water ran off clear, without foam, and let dry at room temperature.

3.3.3 Sonication

During ultrasound treatment cavitation leads to high shear forces on solid particles. Fibres break because of this stress which results in smaller fragments and larger surfaces for enzymes to attach.

Samples were submerged in buffer, cooled with ice and sonicated three times for 5 minutes with an ultrasonic probe (Branson Digital Sonifier model 250). The sonication power was set to up to 50 % amplitude. The fixed frequency of the device was 20,000 kHz. After each sonication step samples were allowed to cool.

3.3.4 Alkaline treatment

NaOH treatment swells cellulose fibres and activates them for subsequent enzymatic hydrolysis. But since PET is sensitive to alkaline treatment at higher temperatures, bot temperature and concentration of NaOH were kept low in these experiments.

PET fibres were treated with varying concentrations of NaOH (0.1 M, 0.5 M and 1.0 M). As only a limited amount of cotton fibre was available these samples were just treated with 0.5 M NaOH. Salesianer 0.5 mm samples were treated with 0.1 and 0.5 M NaOH. The treatment was carried out for one hour at 50 °C in an ultrasonic bath (Ulsonix Proclean 3010, 42 kHz, 300 W) to speed up the reaction. After the treatment NaOH was removed by filtration and the samples were washed with water until their pH was neutral.

3.3.5 Steam explosion

During steam explosion samples are subjected to hot steam and high pressures followed by a sudden decompression. The reduction of the pressure opens fibre structures, potentially facilitating subsequent hydrolysis.

Steam explosion was carried out at Johannes Kepler University Linz, the reactor can be seen in Figure 15. Up to 350 g of textile samples were placed into a reactor and heated up to 150 °C by introducing steam at 5 bar. Residence times of 10 and 15 minutes were tested before opening the decompression valve. Between batches the reactor was flushed multiple times with 180 °C hot steam. After steam explosion samples were transported back to Tulln where further experiments were performed the same day. The condensed steam was analysed via HPLC for glucose.

Figure 15: Steam explosion reactor at Johannes-Kepler-University, Linz. Samples are loaded into one of the two chambers (A) at the side. Once samples were treated at a given pressure, pneumatic valves open and samples are trough piping into the collecting container in the centre (B).

3.4 Enzymatic degradation of cotton

The aim of the cotton hydrolysis is to degrade the cotton component efficiently to yield pure PET fibres which can be re-introduced into textile industry. Therefore, effects of different pre-treatment methods and changing process parameters were investigated with the goal to improve the hydrolysis.

3.4.1 Effects of Cyrene and agitation

Different agitation methods were analysed for their effectiveness in mixing the hydrolysis slurry. Two experiments were conducted with washed denim fibre as substrate using an orbital shaker in the first and a magnetic stirrer plate in the second experiment. Additionally, the effects of the aprotic solvent dihydrolevoglucosenone (Cyrene) were examined. Cyrene acts as a hydrogen-bond acceptor and could swell cellulose fibres facilitating the hydrolysis.

1 g denim sample were each suspended in a solution containing 1 % cellulases (Cellic CTec3) and different concentrations of Cyrene (0, 1.0, 2.5 and 5.0 % v/v) in citric buffer adding up to a total volume of 75 ml per gram textile. Flasks containing the suspensions were then placed on a magnetic stirrer plate or an orbital shaker respectively and incubated for one week at 50 °C. Samples were taken after defined time points for subsequent analysis with HPLC. After the hydrolysis solids were filtered using a metal sieve, washed with purified water and dried for the weight loss analysis.

All subsequent experiments were agitated by magnetic stirrers.

3.4.2 Effects of sample size

Samples of different size were degraded enzymatically to determine whether mechanical pre-treatment provides any benefits for subsequent hydrolysis. Therefore, the hydrolyses of Salesianer uncut, 3 mm and 2 mm samples were compared.

0.267 g textile were suspended in 20 ml citric buffer containing 200 µl cellulase cocktail. All flasks were incubated in an oven at 50 °C. Samples for HPLC were taken at regular intervals. After 8 days solid residues were washed, filtered through a metal sieve and dried for FTIR- and weight loss analysis.

3.4.3 Production of glucose with enzyme recovery

A quick hydrolysis was performed in a 2-litre bottle to provide glucose solutions for a subsequent fermentation. 20 g Salesianer (0.5 mm) were suspended in 1.98 l citric buffer

and 20 ml cellulase cocktail (NS 52 143). The mixture was incubated at 50 °C for 3 days, while samples for HPLC were taken repeatedly. After the hydrolysis the batch was centrifuged for 30 minutes at 10,956 × g, 4 °C. The supernatant was filtered through paper filters (Whatman grade 1) and frozen until further use. The pellet was washed with MQ-H_2O three times and centrifuged between each washing step. Afterwards fibres were dried and analysed with FTIR. Samples of these PET fibres were sent to project partners for mechanical recycling tests.

After thawing, the supernatant was filtered through two Vivaflow 50 ultrafiltration membranes (Satorius) in series. This, on the one hand, results in a pure glucose solution for subsequent fermentation, but on the other hand concentrates the enzyme in the retentate allowing it to be reused. The purification was monitored via SDS PAGE and the recovered enzyme tested for its activity.

3.4.4 Cotton hydrolysis with ultrasound pre-treatment

1 g of Salesianer (2 mm and 3 mm) samples was mixed with 75 ml citric buffer and sonicated as described in chapter 3.3.3. After sonication 750 µl enzyme were added and the samples were incubated at 50 °C for 5 days. Additionally, a reference, without sonication, was incubated. After hydrolysis solids were filtered through a metal sieve, washed and dried for FTIR measurements.

A larger hydrolysis with ultrasonic pre-treatment was performed with Salesianer (0.5 mm samples). 20 g fibres were mixed with 1.98 l citric buffer and 20 ml cellulase and the batch was incubated for 5 days at 50 °C. Glucose concentrations were analysed via HPLC. After the hydrolysis the batch was centrifuged for 30 minutes at 10,956 × g, 4 °C and the supernatant was filtered through paper filters. The pellet was washed with MQ-H_2O, dried and the purity was analysed via FTIR.

The supernatant purified via ultrafiltration to be used in following fermentations.

3.4.5 Effects of ultrasound on cotton hydrolysis

Ultrasound is reported to enhance enzymatic hydrolysis of cotton due to multiple effects. First ultrasound improves mass transfer in the mixture by induction of turbulent flows or cavitation. Secondly the energy introduced via ultrasound is thought to lead to structural changes within the enzyme which facilitate the formation of an enzyme-substrate-complex.

To investigate whether the hydrolysis benefits from ultrasonic treatment, 1 g of bed sheet was suspended in 75 ml citric buffer and 1 % enzyme and incubated at 50 °C for 7 days. 3

and 6 hours after the start of the hydrolysis bottles were exposed to ultrasonic treatment for 20 minutes in the ultrasound bath (Ulsonix Proclean 3010). The treatment was repeated daily until the end of the hydrolysis. For comparison of the different Cellic CTec3 batches one hydrolysis was performed using the HS batch and one using the EU batch respectively.

3.4.6 Cotton hydrolysis with Cyrene

A 2-litre hydrolysis with 1 % Cyrene addition was conducted with subsequent purification of the supernatant for fermentation. Consequently 20 g Salesianer (0.5 mm) were mixed with 1.96 l citric buffer and 20 ml of each Cyrene and enzyme. The hydrolysis was run for 7 days at 50 °C with samples for glucose quantification being taken regularly. After 7 days fibres were separated by centrifugation (30 minutes, 10,956 × g, and 4 °C) and filtration through paper filters. They were washed with water, dried and analysed via FTIR.

The supernatant was purified via ultrafiltration for subsequent fermentations.

3.4.7 Continuous removal of hydrolysis products

To prevent enzyme inhibition by glucose and potentially increase the hydrolysis speed a reactor was designed in which the supernatant was filtered continuously through a Vivaflow 50 ultrafiltration membrane (Figure 16). Thereby smaller hydrolysis products were removed from the reactor while enzymes were fed back into it. The reactor was filled with 20 grams of fibre suspended in 1.98 litres of pre-heated buffer and placed inside a heat-controlled chamber where 20 ml of cellulase were added. The temperature was maintained at 50 °C by a temperature controller (Heju Juchhein Solingen) driving a heating tape. Within the reactor a thin PET mesh was installed to prevent solids of blocking the outflows. The permeate-flow through the ultrafiltration membrane was 10 ml/h and new citric buffer was fed to the reactor with the same rate. Both streams were driven by the same peristaltic pump (Ismatec). Filtrate and buffer feed were kept on ice during the hydrolysis. Glucose concentrations in the permeate and the reactor were monitored via HPLC. After hydrolysis fibres were washed, dried and analysed via FTIR.

Figure 16: Setup of the membrane-reactor. Hydrolysis takes place in the reactor within an insulated, heated chamber. Supernatant is drawn from the bottom of the reactor and transported to an ultrafiltration membrane by a peristaltic pump. The UF-membrane and bottles for buffer and permeate are stored on ice inside the white poly-styrene boxes.

3.4.8 Hydrolysis of steam-exploded samples

In a preliminary test, different steam explosion parameters and their influence on subsequent cotton hydrolysis were tested. Residence times of 10 and 15 minutes were compared to samples without steam explosion treatment. 1 g of each textile sample was mixed with 75 ml citric buffer and 750 µl cellulase and incubated for 7 days at 50 °C. Textiles used for these tests were coarsely cut Salesianer, since no 0.5 mm fibres were available at that time.

Afterwards, Salesianer (0.5 mm) samples were treated with steam explosion (150 °C, 10 minutes) and subjected to 16 hours of alkaline treatment with 1 M NaOH over night at 4 °C. Samples were washed with water until the pH was neutral and excess water was removed by squeezing. 60 g wet fibres (corresponds to approximately 20 g dried fibres) were mixed with 1.96 l citric buffer and 40 ml cellulase and incubated at 50 °C. After one week the supernatant was removed by filtration and solids were washed with water. Fibre residues were additionally treated with 3 M solution for one hour at 50 °C. Finally, fibres were washed with water, dried and the purity was examined via FTIR. Samples of purified PET fibres were sent to project partners for mechanical recycling.

The supernatant was purified via ultrafiltration and sterilized by filtration through a 0.2 µm sterile-filter.

3.5 Enzymatic degradation of PET

Although this project's goal is to recycle PET a lot of synthetic fibres are disposed of improperly and end up in organic waste. Therefore, the biological degradation of those is of interest too and was investigated in this thesis.

Washed and dried PET-residues from cotton hydrolysis were degraded using cutinase. 9 g PET were suspended in 981 ml K-PO4 buffer and 8.85 ml cutinase (0.1 mg cutinase per ml total volume). The mixture was incubated for 7 days at 60 °C. Samples were taken in regular intervals and analysed for terephthalic acid and BHET release with HPLC. After hydrolysis solids were washed, dried and measured with FTIR.

3.6 Ethanol fermentation

3.6.1 Preculture

50 ml YPD-medium were inoculated with one culture of *S. cerevisiae* taken from an agar plate. The pre-culture was incubated overnight for 19 hours at 28 °C, 100 rpm.

3.6.2 Ethanol fermentation of glucose from hydrolysis

Yeast and peptone were dissolved in the cotton hydrolysate prepared in chapter 3.4.3. 1.25 l medium were inoculated with 50 ml preculture (OD_{600} = 2.071). The flask incubated for 3 days at 28 °C and 120 rpm. Samples were taken regularly and analysed for glucose and ethanol concentrations and cell growth (OD_{600}).

3.6.3 Comparing glucose sources for fermentation

To see whether glucose from hydrolysis was used as readily as commercial glucose by *S. cerevisiae*, a 3.65 g/l solution of D-glucose was prepared, to match the concentration of the cotton hydrolysate prepared in chapter 3.4.4.

YPD-media were prepared with two concentrations of glucose whereby glucose by either of the two solutions. YPD media containing 0.2 % glucose were prepared by mixing 82 ml glucose and 67 ml yeast-peptone-medium and 61.2 ml glucose solution were mixed with

87 ml yeast-peptone-medium to create 0.15 % glucose media. All flasks were inoculated with 1 ml pre-culture and incubated for 3 days at 28 °C 120 rpm. Glucose and ethanol concentrations were measured at regular intervals.

3.6.4 Aerobic and anaerobic ethanol fermentation

Ethanol production under aerobic and anaerobic conditions was compared to each other. Additionally, it was tested if the presence of Cyrene in the media has an influence on the fermentation. Therefore YPD-medium was prepared from regular cellulose hydrolysate (chapter 3.4.3) and hydrolysate containing Cyrene (chapter 3.4.6).

50 ml medium were inoculated with 1.6 ml preculture and incubated for 3 days at 28 °C and 150 rpm.

While the aerobic fermentation was carried out in sterile 100 ml Erlenmeyer flasks, the anaerobic fermentation was performed in sterile 100 ml crimp-sealed bottles. To avoid pressure formation in anaerobic fermentations sterile gas sampling bags were attached. After Medium and inoculum were added to the anaerobic bottles the suspension was sparged with N_2 for 30 seconds to remove oxygen.

Glucose and ethanol concentrations were monitored via HPLC and cell growth was analysed via optical density measurements (OD_{600}).

3.7 Algae cultivation in cellulose hydrolysate

Two flasks containing 95 ml sterilised cellulose hydrolysate (produced in chapter 3.4.8) was inoculated with 5 ml *Chlorella sorokiniana* pre-culture each. One of the flasks had 1 g/l $NaNO_3$ added serving as nitrogen source. Algae were incubated at 32 °C, 100 rpm for 8 days. Growth was monitored via optical density.

3.8 *Trichoderma reesei* cultivation in cellulose hydrolysate

To test whether cellulases could be produced wit hydrolysate as carbon source, two 100 ml Erlenmeyer flasks containing 50 ml YPD-medium with glucose from hydrolysis (chapter 3.4.3) were inoculated each with half the lawn from a *T. reesei* agar plate and incubated at 24 °C, 150 rpm for 5 days. The enzyme production was monitored via cellulase activity assay and SDS PAGE.

4 Results

4.1 Enzyme characterisation

4.1.1 Biochemical characterization of enzymes.

Protein concentrations and activities of all three cellulase mixtures were compared and their composition was analysed via SDS PAGE. While protein concentrations were very similar, there were notable differences in enzyme activity. NS 52 143 is the most active with 2280 U/ml. Cellic CTec3 variants fall behind with EU being the least active (Table 4). The enzyme mixtures seem to be composed of the same proteins as they have identical bands on SDS PAGE. NS 52 143 has a more intense band at 130 kDa and the band at 30 kDa is weaker (Figure 17).

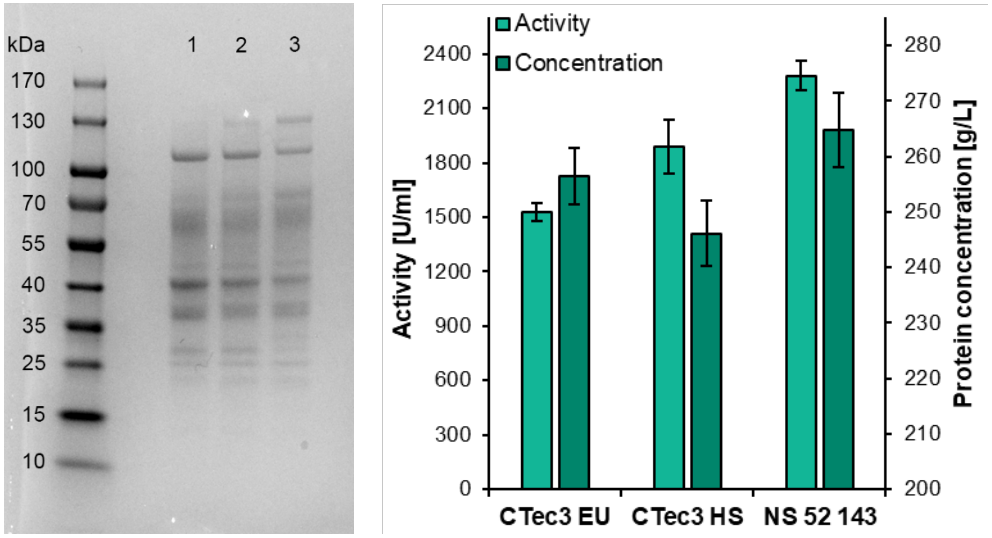

Figure 17: Characterisation of cellulases. Left: SDS PAGE of different cellulase mixtures: (1) Cellic CTec3 EU, (2) Cellic CTec3 HS and (3) NS 52 143. Right: comparison of the three cellulase mixtures in regards of activity (U/ml) and protein concentration (g/l).

Table 4: Protein concentrations and activities of the three cellulase mixtures NS 52 143, Cellic CTec3 EU and Cellic CTec3 HS.

Enzyme	Concentration [g/l]	Activity [U/ml]	Spec. activity [U/mg]
NS 52 143	264.77 ± 6.81	2280.36 ± 79.55	8.89 ± 0.486
Cellic CTec 3 HS	246.11 ± 5.82	1887.60 ± 146.91	7.67 ± 0.778
Cellic CTec 3 EU	256.50 ± 5.06	1530.57 ± 49.95	5.78 ± 0.337

The cutinase used for degradation of PET had an activity of 1136 U/ml. The protein concentration, determined via Bradford method was 10.17 ± 0.51 g/L.

4.1.2 Cellulase stability in Cyrene solution

Incubation of cellulases in citric buffer containing Cyrene leads to a decrease of activities in comparison to samples where no Cyrene was added. The enzyme blank was most active at the start of the experiment. After 3 hours the activity dropped between 1300 to 1550 U/ml where it stayed stably for the rest of the incubation. In the presence of Cyrene, activities were about 50 % lower at the start and further decreased to under 15 U/ml after one week. Varying the concentration of Cyrene from 1 % to 2.5 % did not make a difference during the incubation (Figure 18).

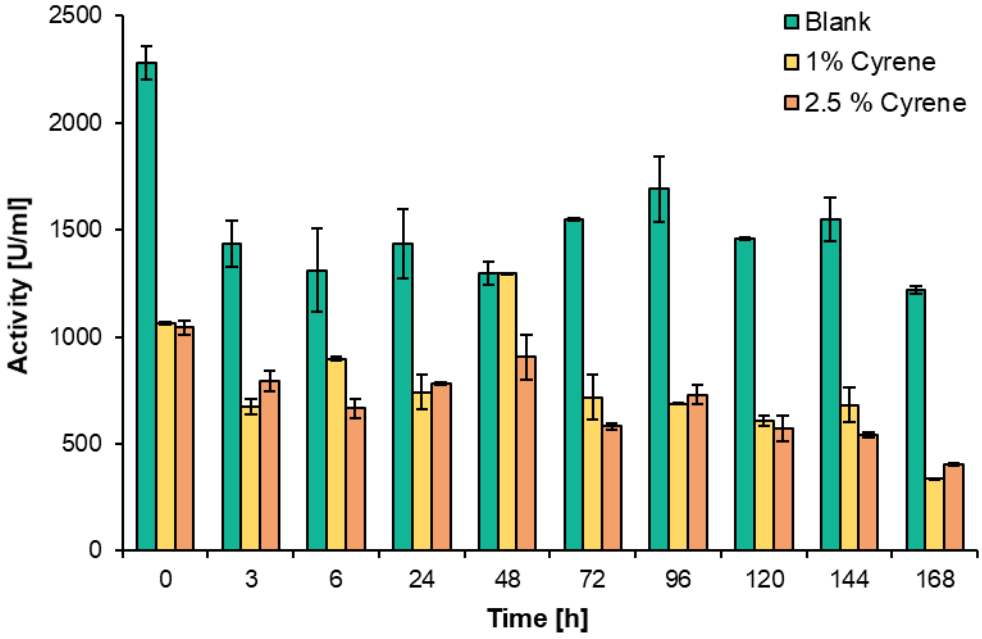

Figure 18: Comparison of cellulase stability with and without addition of Cyrene.

4.1.3 Influence of ultrasound on cellulase activity

Repeated sonication of enzyme solutions at 42 kHz and 300 W on average lowered the enzyme activity by 14 % during the stability test compared to the enzyme blank (Figure 19).

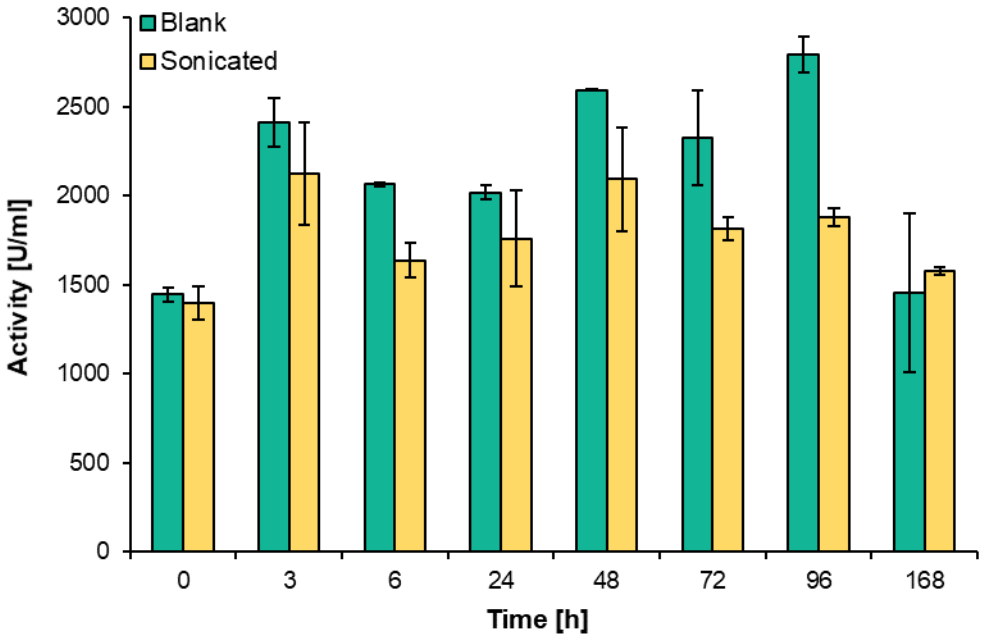

Figure 19: Cellulase stability under the influence of repeated sonication (yellow) compared to a blank of untreated enzyme (green).

4.2 Pre-treatment

4.2.1 Ultrasonic- and alkaline pre-treatment

During pre-treatment with NaOH and ultrasound terephthalic acid concentrations and weight loss were determined to observe the effects the treatment has on pure PET and mixed fibres. Effects of ultrasonic pre-treatment on cotton were analysed via HPLC and weight loss. Glucose release of alkaline-treated samples could not be measured via HPLC as only one large unidentifiable peak eluted during analysis.

Pure PET was hydrolysed by NaOH in small amounts. The higher the concentration of NaOH, the more terephthalic acid was released. The same was observed when measuring terephthalic acid (TA) concentrations from Salesianer samples after alkaline treatment (Figure 20).

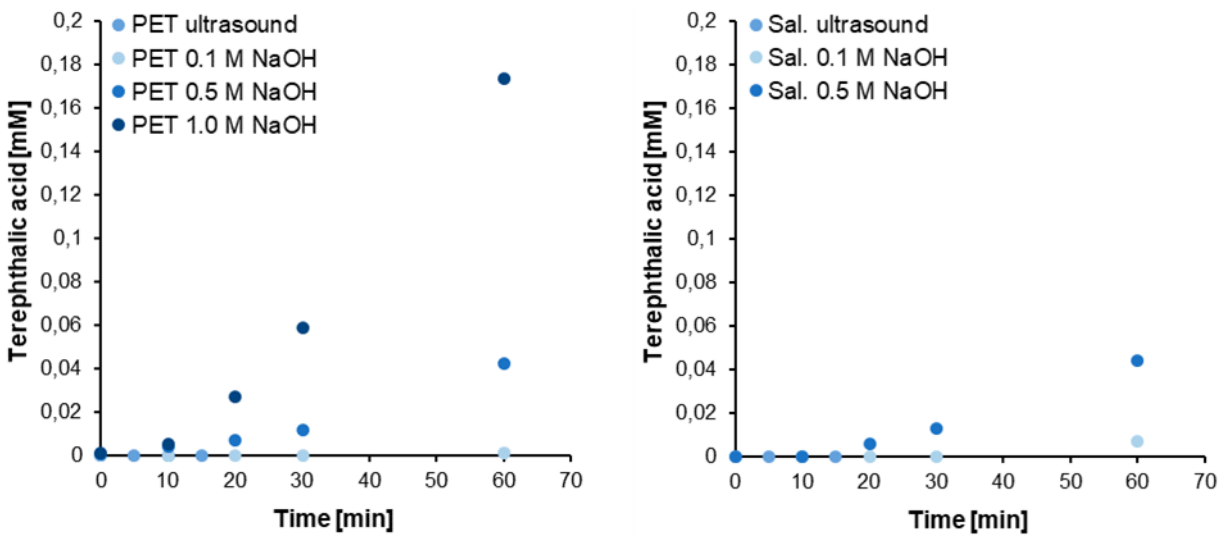

Figure 20: Terephthalic acid release from PET (left) and Salesianer samples (right) during pre-treatment with ultrasound and NaOH of varying concentrations.

While terephthalic acid release was in the µM range and therefore not quantifiable via weighing, the degradation of cotton, and therefore of mixed fibres as well, during alkaline treatment is measurable via weight analysis (Figure 21). Treatment of cotton with NaOH leads to degradation and swelling of the fibre, potentially facilitating subsequent enzymatic hydrolysis. Degradation of PET was neglectable after 1-hour treatments making alkaline treatment a suitable pre-treatment for cotton-PET-fibres. Neither cotton nor PET were degraded by ultrasonic pre-treatment, hence no terephthalic acid or glucose were detected.

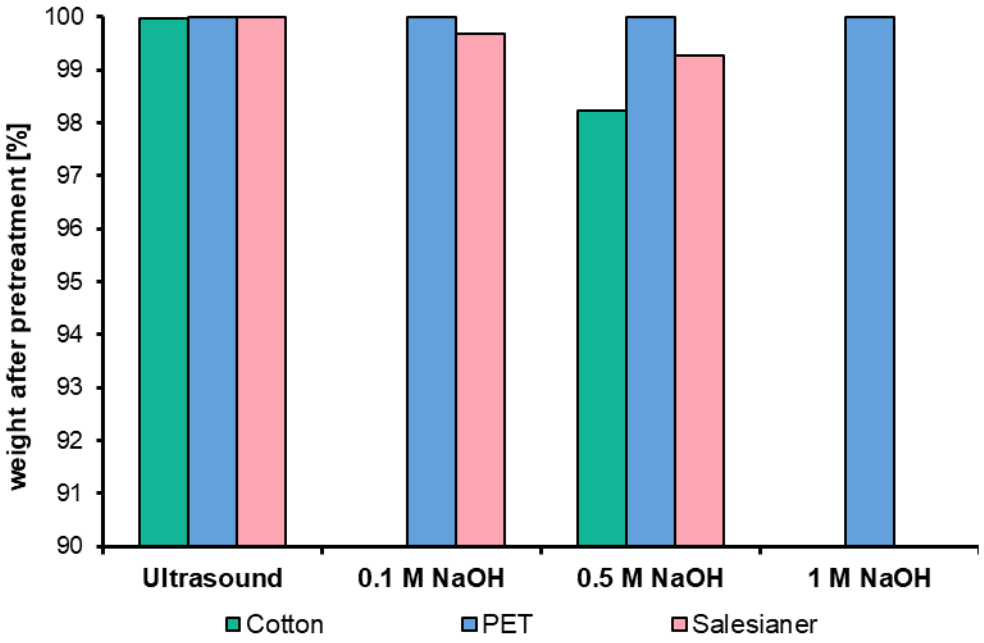

Figure 21: Weight loss of cotton, PET and Salesianer textiles during pre-treatment experiments.

4.2.2 Steam explosion

During HPLC analysis of condensed steam no glucose was found, therefore no degradation of cotton took place during steam explosion. The main goal of such treatment is to loosen the structure of cotton, so that enzymes can move within the network and attach to fibrils. After steam explosion textile samples had visibly increased volume, making the desired effect evident (Figure 22).

Figure 22: 100 g of coarse textile samples before (left) and after (right) steam explosion.

4.3 Enzymatic degradation of cotton

4.3.1 Effects of Cyrene and agitation

The effects of mixing became very clear during the hydrolysis. Samples on the orbital shaker were visibly degraded less than samples on a magnetic stirrer, which is also reflected on the weight loss analysis (Figure 23).

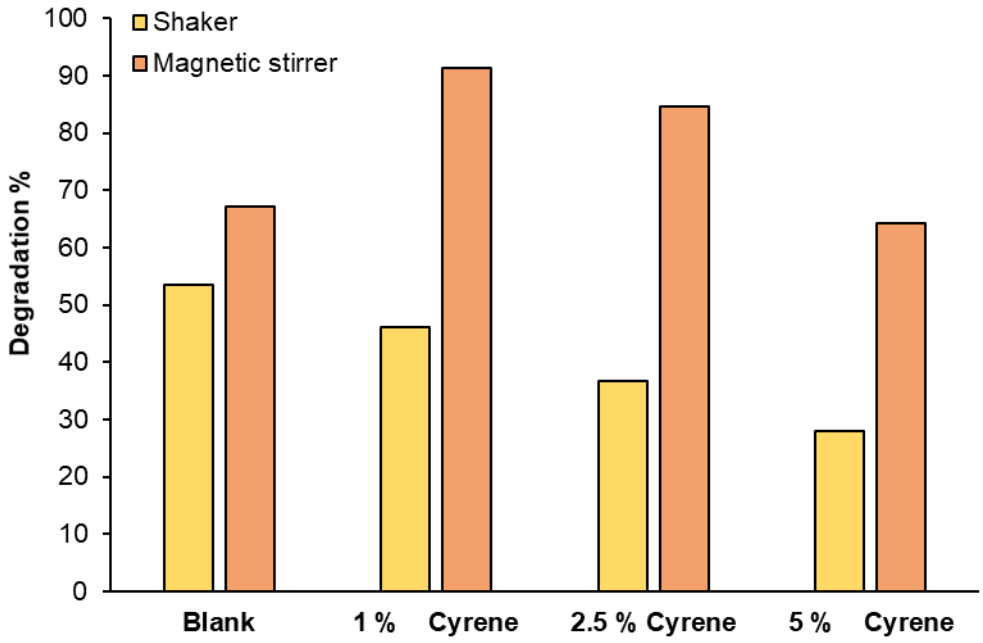

Figure 23: Weight loss of Denim fibres after enzymatic hydrolysis under the influence of Cyrene and different agitation methods.

Cyrene had different effects on the samples, depending of the agitation method. Hydrolyses incubated on the orbital shaker were affected negatively by the solvent. The poor mixing probably had multiple effects: Enzymes could not diffuse into the cellulose network but were only able to attack cellulose at the surface or at fibres' ends. Cyrene, as well, could not move into the network to open up its structure, but disturbed enzymes in the slurry as observed in the stability tests (chapter 4.1.2).

When agitated by a magnetic stirrer, hydrolysis overall was better. Addition of 1 % Cyrene further improved the degradation of cotton, resulting in a weight loss of 90 % after one week of incubation. The solvent helped loosen crystalline cellulose structures, facilitating enzymatic degradation in those areas. When more Cyrene was used, its negative effects became more dominant resulting in poorer degradation.

Addition of 1 % Cyrene also resulted in a glucose concentration of 6.1 g/l which is the highest concentrations of samples incubated on the magnetic stirrer. Interestingly the enzyme blank which was incubated on the orbital shaker released the most glucose (7.3 g/l), even though the overall degradation was poorer (Figure 24). It seems like agitation supports the overall degradation and cutting of cellulose strands into smaller particles while static hydrolysis promotes glucose generation.

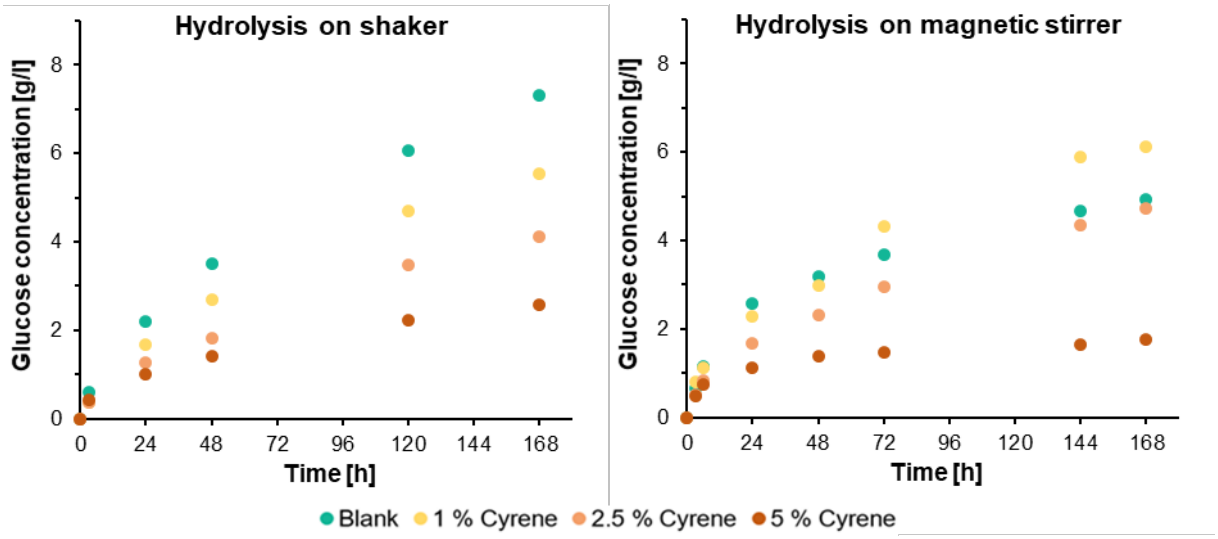

Figure 24: Glucose release of Denim fibres during the hydrolysis on an orbital shaker (left) or a magnetic stirrer (right). The influence of Cyrene on the hydrolysis can be seen.

The role of Cyrene becomes evident when comparing the enzyme blank on the magnetic stirrer to the respective 2.5 % Cyrene sample. Even though the conversion of cotton to glucose was similar, the overall weight loss was better when Cyrene was added. Cyrene opened the complex crystalline structure of cellulose allowing endoglucanases to cut cellulose strands and therefore reducing the strand length until they became either soluble or small enough to pass the sieve during subsequent filtration. Exoglucanases, which release cellobiose from strands' ends, and glucosidases, which convert cellobiose to glucose, did not benefit from Cyrene, but only faced its negative effects.

4.3.2 Effects of sample size

The smaller fibres were cut during mechanical pre-treatment, the better the enzymatic degradation progressed. 2 mm samples released most glucose (3.4 g/l), followed by 3 mm fibres (3.0 g/l) and uncut samples, which released the least glucose. Weight loss analysis too reflected the outcome. Uncut samples only lost 27 % of their weight while 3 mm samples lost 34 %. 2 mm samples were degraded most. The weight loss result of 2 mm samples,

however, does not reflect the degradation. As the metal sieve was too coarse for fibres of this size, some were lost during the washing process, distorting the result (Figure 25).

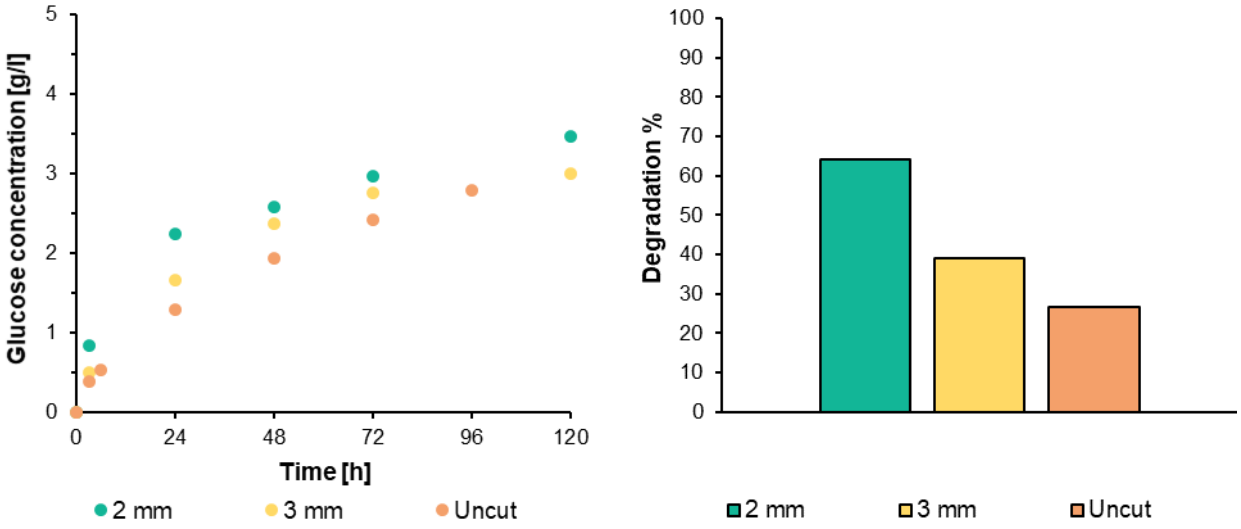

Figure 25: Influence of sample size on the glucose release (left) and weight loss (right) after enzymatic treatment.

4.3.3 Production of glucose with enzyme recovery

After 72 hours of hydrolysis the glucose concentration reached 3.0 g/l (Figure 26 left) and the weight loss was 35 %. After the additional washing step with urea the weight loss could be raised to 42 % so that most of the cotton was removed. Nevertheless, cotton residues could still be seen during FTIR analysis. Both the hydroxyl peak at 3350 cm^{-1} and the C-O stretch of cotton (1020 cm^{-1}) are clearly visible (Figure 26 right).

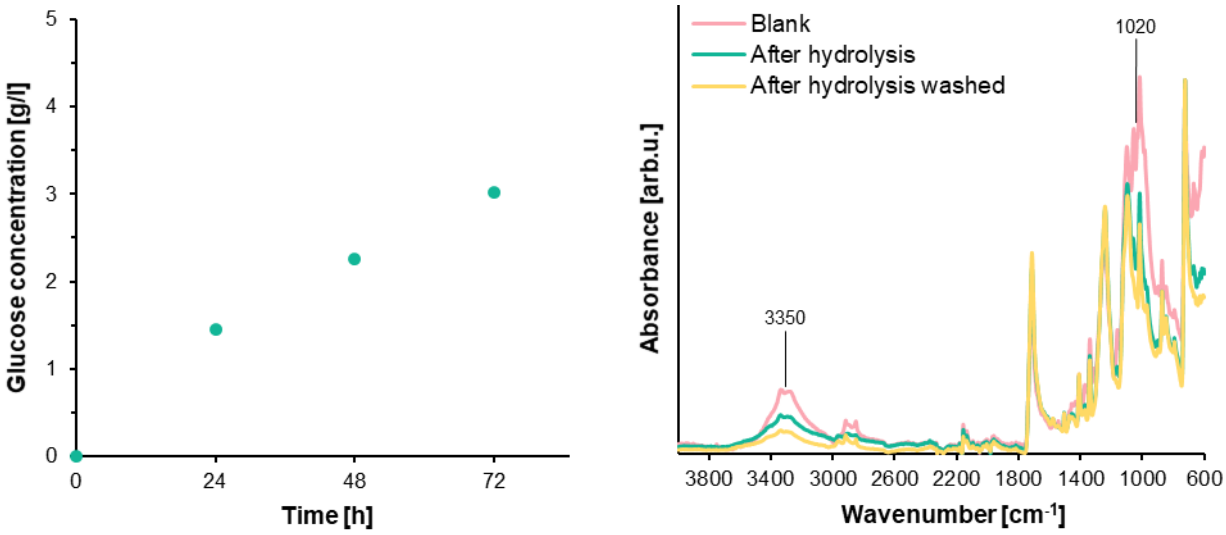

Figure 26: Left: glucose release during the 72 hour hydrolysis. Right: FTIR spectra of fibres after hydrolysis and after an additional washing step compared to a fibre blank.

There were still some brown impurities in the otherwise white PET, but apart from the colour it had similar properties and could be processed like virgin PET as project partners demonstrated by melting the material into pellets and spinning fibres thereof (Figure 27).

Figure 27: Left: purified PET after cotton hydrolysis. Right: mechanically recycled PET pellets and fibres from purified PET.

The ultrafiltration process removed all enzymes from the glucose solution which could then further be used for fermentations. The purification was verified via SDS PAGE. While bands in the concentrate (C) are more pronounced than they are in the feed (F), the permeate (P) did not contain any proteins (Figure 28 left).

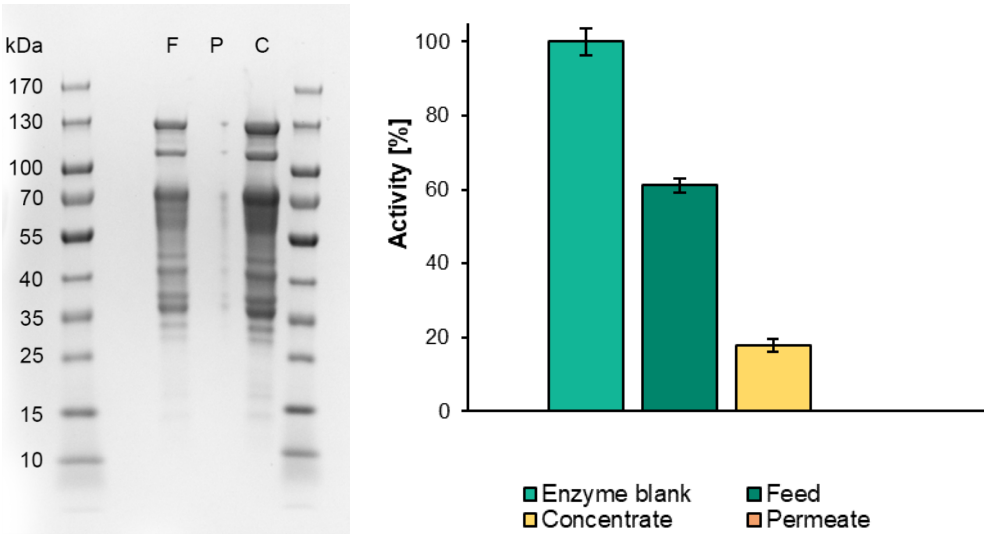

Figure 28: Results from enzyme recovery. Left: SDS PAGE of ultrafiltration streams; F: feed, P: permeate, C: concentrate. Right: Cellulase activity measured in ultrafiltration streams.

During the hydrolysis the enzyme activity decreased to 60 % of its initial activity, which is in accordance to the stability test (chapter 4.1.2). The enzyme concentrate obtained through ultrafiltration only had 33 % of cellulase activity (Figure 28 right). Some of the enzymes were stuck on the membrane and the fibres and washed out with the washing water, resulting in enzyme loss, which explains the decreased activity.

It is possible to recover the enzyme with ultrafiltration, but the process needs to be improved so that the recovery rate is higher. If the enzyme was to be used for another hydrolysis, fresh enzyme solution needs to be supplemented to reach the same activity during hydrolysis.

4.3.4 Cotton hydrolysis with ultrasound pre-treatment

HPLC analysis shows a small increase in glucose concentration of sonicated 2 mm samples (3.5 g/l), compared to the 2 mm blank (3.4 g/l). The difference is more pronounced during the first days of the hydrolysis than it is at the end, when the plateau is reached. As the same sieve as before was used, some smaller particles were lost when fibres were washed, resulting in high weight loss of 2 mm samples.

Ultrasonic pre-treatment of 3 mm samples resulted in a poorer hydrolysis than its respective blank. Additional to a worse weight loss, less glucose was released too (Figure 29).

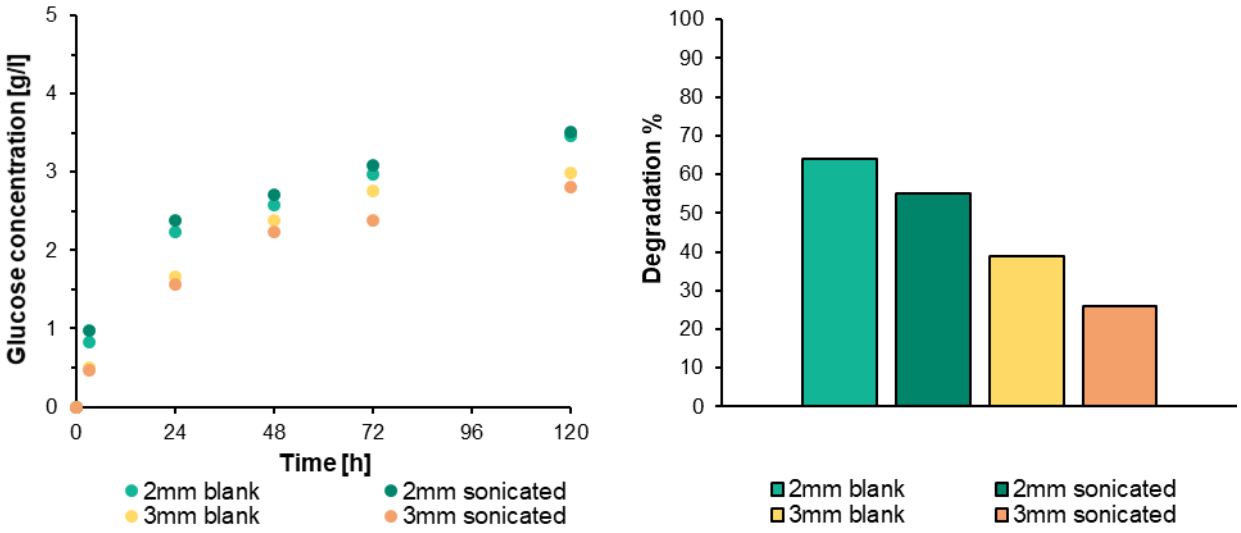

Figure 29: Glucose release (left) and weight loss results (right) of the hydrolysis of 2 and 3 mm samples with ultrasonic pre-treatment.

During FTIR analysis of hydrolysis samples a negative peak occurred at 3300 cm^{-1}, which was probably due to an operator error.

Sonicated and non-sonicated PET-residues of 2 mm samples were of similar purity. The C-O stretches of cotton disappeared and only the distinct twin peaks of PET remained at 1100 and 1015 cm^{-1} (Figure 30 left).

Sonicated 3 mm samples had higher cotton impurities than the 3 mm blank, which is visible in the area at 1020 cm^{-1} (Figure 30 right).

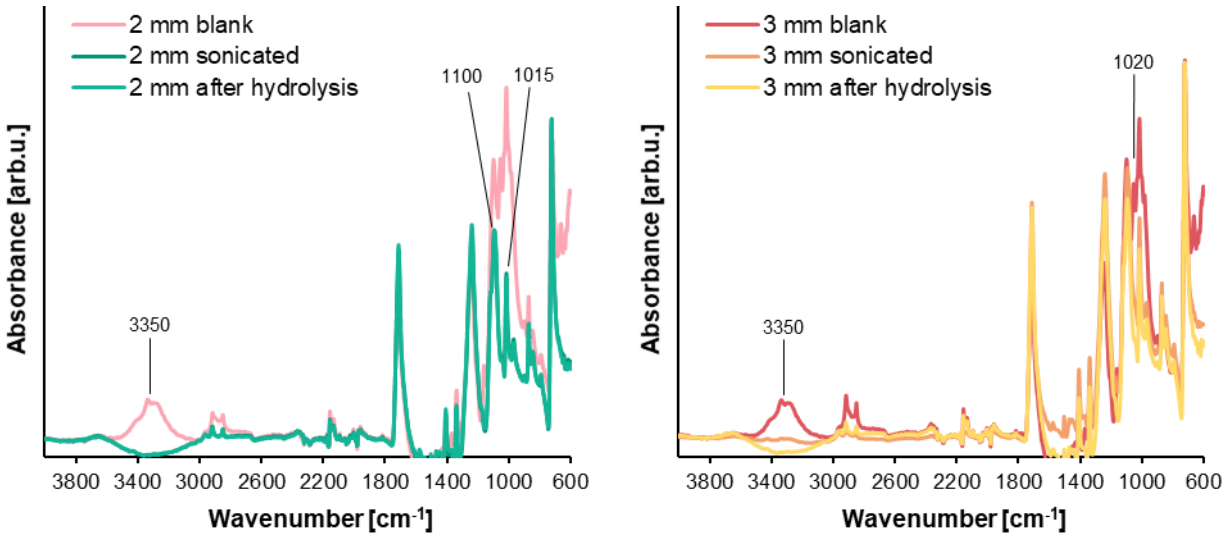

Figure 30: FTIR spectra of hydrolysed 2 mm (left) and 3 mm (right) samples with and without ultrasonic pre-treatment.

Since the weight loss of 2 mm samples was not reliable and glucose quantification indicated better degradation of cotton than it was observed with 3 mm samples, the particle size seems to have an influence on the effects of ultrasonic pre-treatment. Therefore, a larger hydrolysis was performed using Salesianer 0.5 mm samples, as soon as they were available.

The hydrolysis of 0.5 mm fibres yielded a glucose concentration of 2.8 g/l (Figure 31 left). At first this might seem less than the hydrolysis with 2 mm samples, but it has to be considered, that the fibre to buffer ratio was different in small and big batches. In small batches 1 g fibre was incubated in a volume of 75 ml, while in large batches 10 g textile were suspended in a volume of 1 litre, which is 25 % less fibres per volume. Therefore, glucose concentrations in big batches are expected to be 25 % lower than in small batches, which is the case in this experiment.

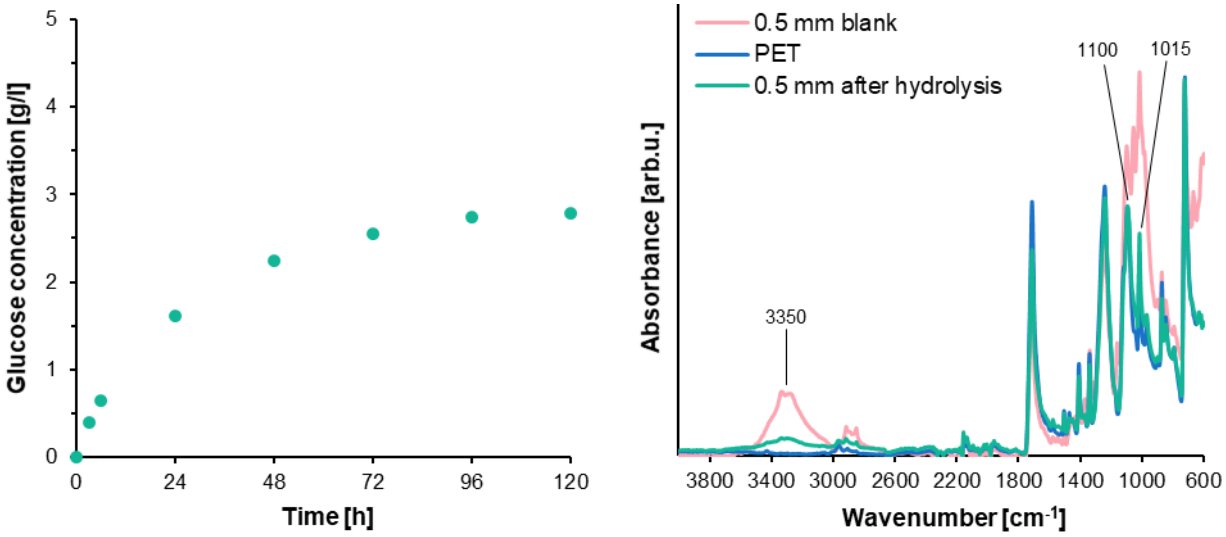

Figure 31: Results from hydrolysis of ultrasound pre-treated 0.5 mm samples. Left: glucose release of during hydrolysis. Right: FTIR spectrum of sonicated textile samples compared to a textile blank and pure PET.

Weight loss of this hydrolysis was 42.7 %, suggesting that about 3 % cotton were left, which is also reflected in the FTIR spectrum. While the characteristic PET peaks at 1100 and 1015 cm^{-1} are already very distinct, the hydroxyl peak around 3300 cm^{-1}, derived from cotton, is still visible (Figure 31 right).

After 5 days of hydrolysis a brown powder was visible in the bottle alongside white PET (Figure 32). These remnants of cotton were separated from PET by sedimentation and filtration and analysed via FTIR as well. The spectrum shows typical O-H (3350 cm^{-1}) and C-O stretches (1020 cm^{-1}) of cotton. N-H bending at 1628 cm^{-1} and N-O stretching at 1525 cm^{-1} indicate the presence of enzymes (Figure 33).

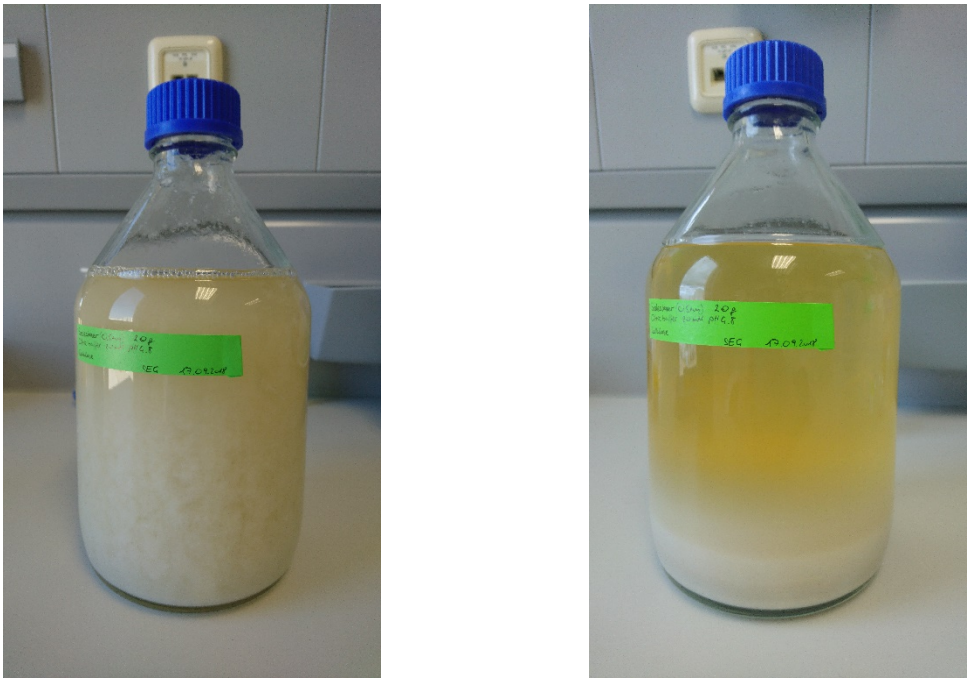

Figure 32: 0.5 mm textile samples in the enzyme mixture at the start of the hydrolysis (left) and after 5 days (right). After 5 days PET is visible as white powder at the bottom of the bottle. Cotton residues sedimented on top as brown powder.

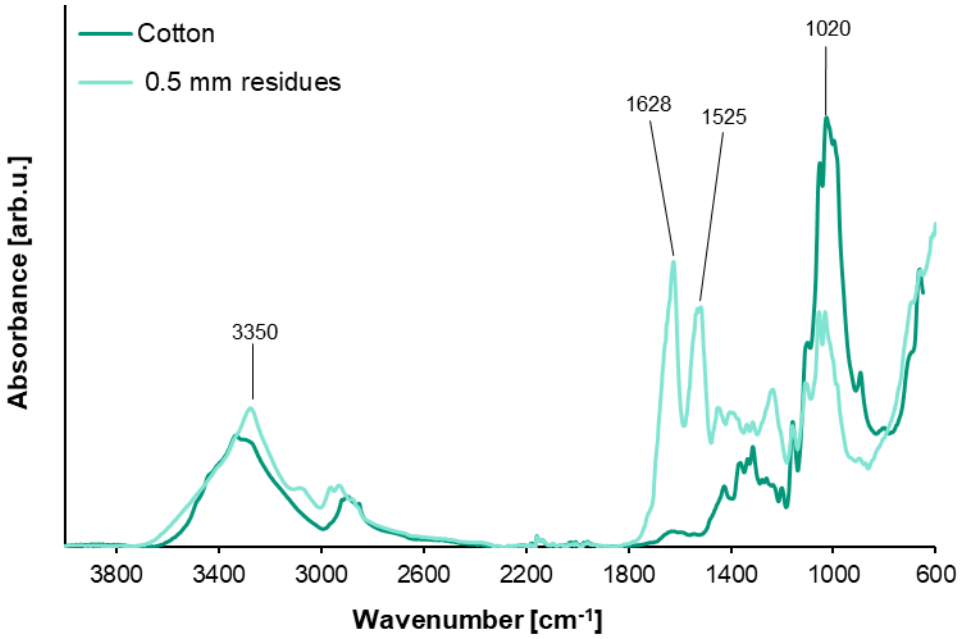

Figure 33: FTIR spectrum of brown cotton residues after hydrolysis of 0.5 mm textile samples compared to pure cotton.

4.3.5 Effects of ultrasound on cotton hydrolysis

In chapter 4.1.3 it was discovered, that repeated sonication reduced enzyme activity. Applying the same treatment during a cotton hydrolysis resulted in less weight loss and glucose release compared to a hydrolysis without ultrasonic treatment. So recurring ultrasonic treatment during hydrolysis did not benefit the degradation of cotton (Figure 34).

The cellulase mixture Cellic CTec3 EU yielded higher glucose concentrations than the HS batch. Fibres incubated with Cellic CTec3 HS were degraded to a higher degree, as shown by the weight loss analysis.

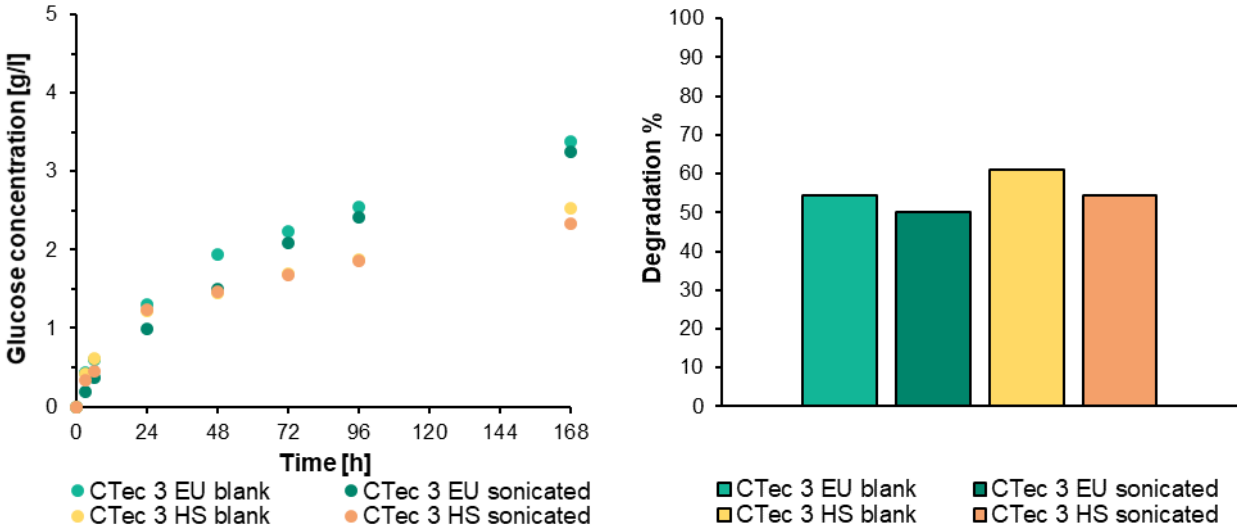

Figure 34: Results of cotton hydrolysis under influence of repeated ultrasonic treatment. Left: glucose release, right: weight loss analysis.

4.3.6 Cotton hydrolysis with Cyrene

After 7 days of hydrolysis with 1 % Cyrene there were still a lot of brown particles in the slurry indicating a poor degradation of cotton (Figure 35). Most of the cotton residues could be removed via sedimentation and filtration so that a weight loss of 45 % could be achieved.

Figure 35: Pictures of cotton hydrolysis with Cyrene addition. Left: Start of hydrolysis. Right: Batch after 7 days of incubation.

The glucose release curve levelled out at 1.7 g/l after 48 hours, which is lower and faster than in hydrolyses where Cyrene was not present. Usually the plateau was reached after 5 days of incubation. After 7 days, the glucose concentration increased to 3.9 g/l, a concentration higher than any other hydrolysis of mixed fibres has yielded so far (Figure 36 left).

The peak of O-H stretches (3350 cm^{-1}) and the C-O stretches of cotton overlapping PET twin-peaks at 1020 cm^{-1} indicated the presence of cotton residues after 7 days of hydrolysis (Figure 36 right). Since cotton residues were already visible in the slurry this was expected.

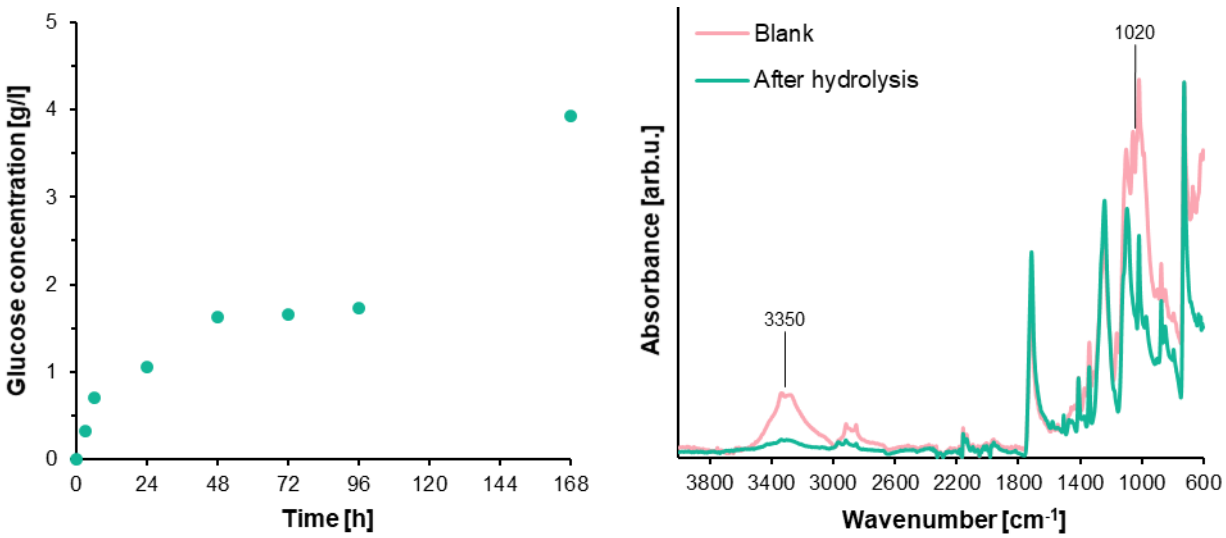

Figure 36: Results of hydrolysis of Salesianer textiles with Cyrene addition. Left: glucose release; Right: FTIR spectrum of fibre residues after hydrolysis compared to a textile blank.

4.3.7 Continuous removal of hydrolysis products

The ultrafiltration had to be stopped after 48 hours, as fibres from the reactor main chamber passed the internal membrane leading to fouling of the ultrafiltration membrane. Nevertheless, the hydrolysis was kept running for 5 more days.

Consequently, 3.30 g fibres with a brown tone were collected from the bottom reactor chamber and 11.84 g white fibres from the main chamber above the membrane. Both fractions were analysed via FTIR separately. Even though the overall degradation was poor with a weight loss of only 24.3 %, the main fraction is very pure PET. The PET twin peaks at 1015 and 1100 cm^{-1} are very well defined and only a minor hydroxyl peak at 3350 cm^{-1} indicates the remains of cotton (Figure 37 left).

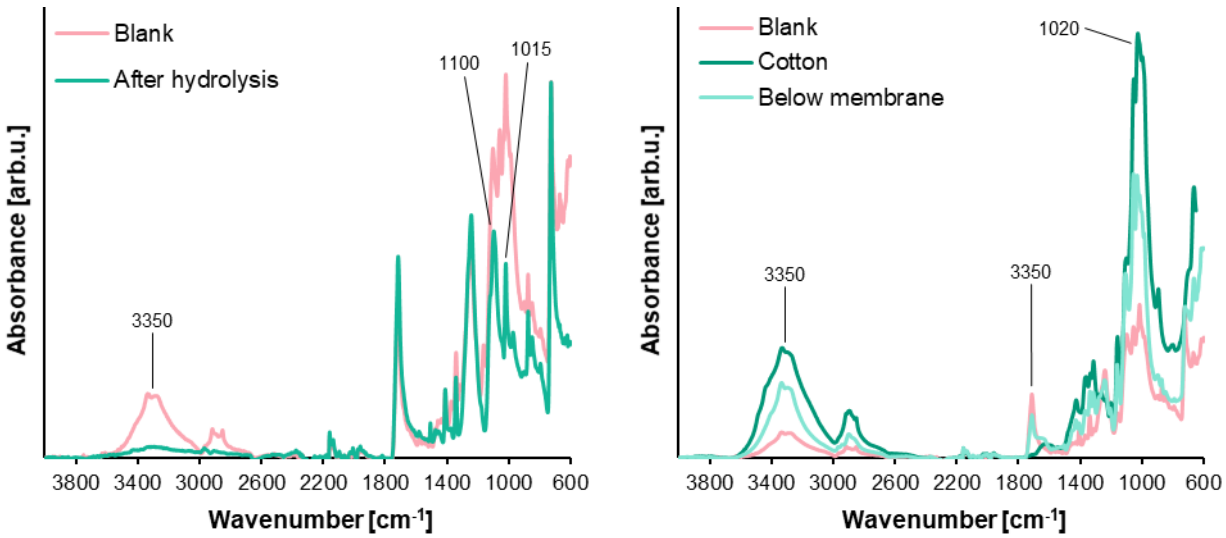

Figure 37: FTIR spectra of residues from hydrolysis in the membrane-reactor. Left: purified PET fibres from the reactor main chamber; Right: fibres collected from underneath the membrane in the bottom of the reactor.

Fibres which passed the membrane and sedimented in the bottom chamber were barely degraded. Interestingly FTIR analysis revealed a higher share of cotton in this fraction, while the PET content was lowered. This is visible on the one hand because of higher O-H and C-O stretches (3350 cm^{-1} and 1020 cm^{-1} respectively) whose intensity is between pure cotton and the Salesianer blank, and on the other hand because of lower intensity of C=O stretches (1700 cm^{-1}) compared to the blank (Figure 37 right).

It seems possible that cellulose fibres which were cleaved by enzymes above the membrane were able to pass the membrane more easily compared to PET fibres which were unaffected by the enzymes. Therefore, the cellulose content below the membrane could increase.

The glucose concentration curve is, apart from two time points, flatter than in previous hydrolyses, with a final concentration of 2.38 g/l (Figure 38). This value represents the total glucose released per volume considering glucose in the permeate and in the reactor. The two outliers occurred as the filtration was stopped. Fibres which migrated to the bottom of the reactor were slowly degraded by enzymes. But as the membrane prevented mixing of the bottom chamber, the released glucose remained near the sampling valve. The same phenomenon as observed in chapter 4.3.1, where highest glucose concentration occurred in poorly agitated batches, arose here as well. Glucose concentration of the last time point was measured after the contents of the bottom and upper chamber were mixed and supernatant and solids were separated from each other.

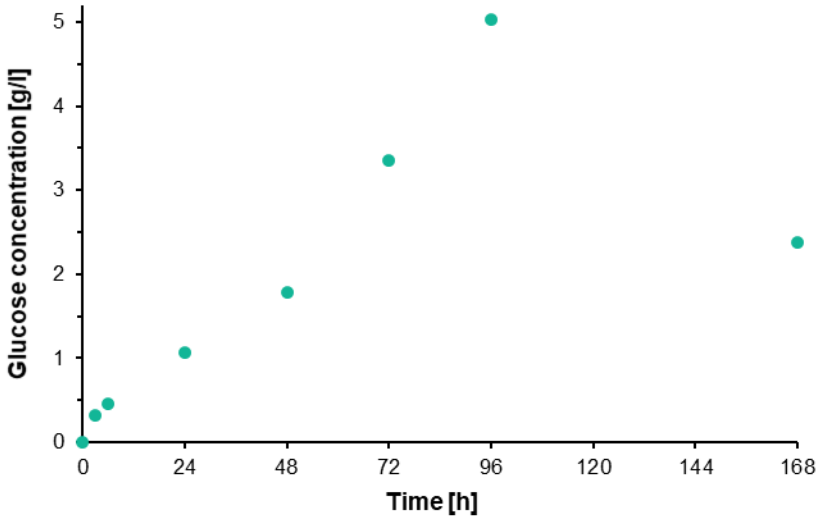

Figure 38: Glucose concentrations measured during the hydrolysis in the membrane-reactor.

Despite the problems which occurred when using the reactor, the products were comparable to conventional batches. The glucose concentration was slightly less compared to other batches, but the fibres collected from the main chamber were among the purest.

The seat of the internal membrane still needs to be sealed and made impermeable to fibres, but since the results were already promising, a more complete hydrolysis is expected once the flaws in the reactor design are mended.

4.3.8 Hydrolysis of steam-exploded samples

In the preliminary test different steam explosion parameters were tested on coarse textile samples. The longer fabrics resided in the steam explosion reactor the less they were degraded during hydrolysis. This result is visible both in weight loss and glucose release (Figure 39). Since the glass transition temperature of PET is around 80 °C, the long incubation time at 150 °C may have caused it to become more viscous and encapsulate cotton fibres so that the enzymes could not bind to the cellulose anymore.

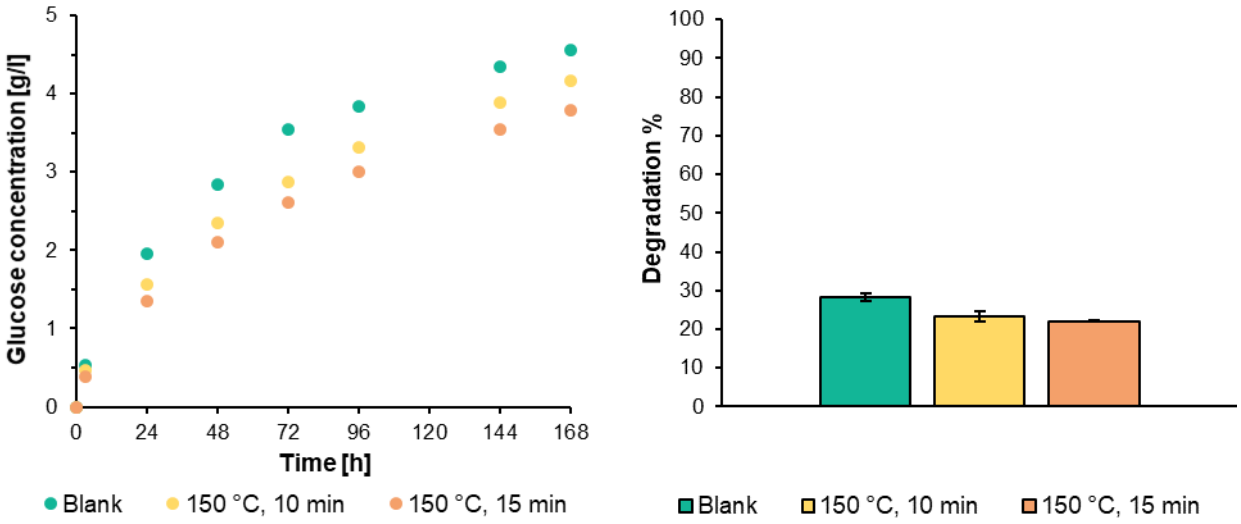

Figure 39: Influence of different steam explosion parameters on the cotton hydrolysis. Left: glucose release. Right: weight loss after 7 days of hydrolysis.

During the following hydrolysis of steam-exploded Salesianer 0.5 mm fibres 3.4 g/l glucose were released after 7 days (Figure 40 left). Considering the different volume-to-fibre-ratio in small and big batches (as mentioned in chapter 4.3.4) higher glucose concentrations were achieved in the big hydrolysis in proportion to the preliminary test. This could be due to the smaller fibre size, the higher enzyme concentration or the additional alkaline pre-treatment.

PET residuals after the hydrolysis and subsequent washing steps were very pure as seen in the FTIR spectrum. All cotton peaks of the samples FTIR spectra were gone and a spectrum identical to pure PET was obtained (Figure 40 right).

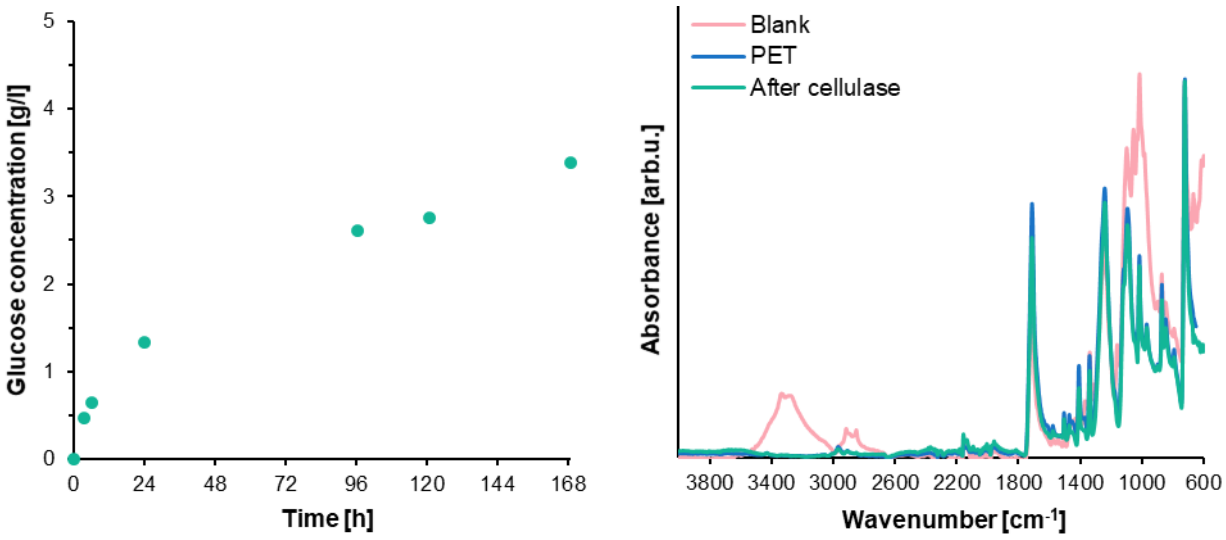

Figure 40: Cotton hydrolysis with steam explosion and alkaline pre-treatment. Left: glucose release. Right: FTIR spectrum of fibre residues compared to a blank and pure PET.

Unfortunately, at the end of this thesis samples from mechanical recycling of these PET residues were not received. But since the FTIR spectrum indicates the highest purity, white PET pellets and yarn were expected. The last recycling experiments already yielded good results, only a slight brown tone indicated impurities.

4.4 Enzymatic degradation of PET

Once cotton was removed the remaining PET fibres were degraded with cutinase. After one week of incubation 3.7 % of the polymer were degraded resulting in a terephthalic acid concentration of up to 1 mM (Figure 41 left). The enzyme first cut off the pre-cursor MHET (mono-2-hydroxyethyl terephthalate) before degrading it to terephthalic acid (TA) as can be seen by comparing the chromatograms of different time points (Figure 41 right).

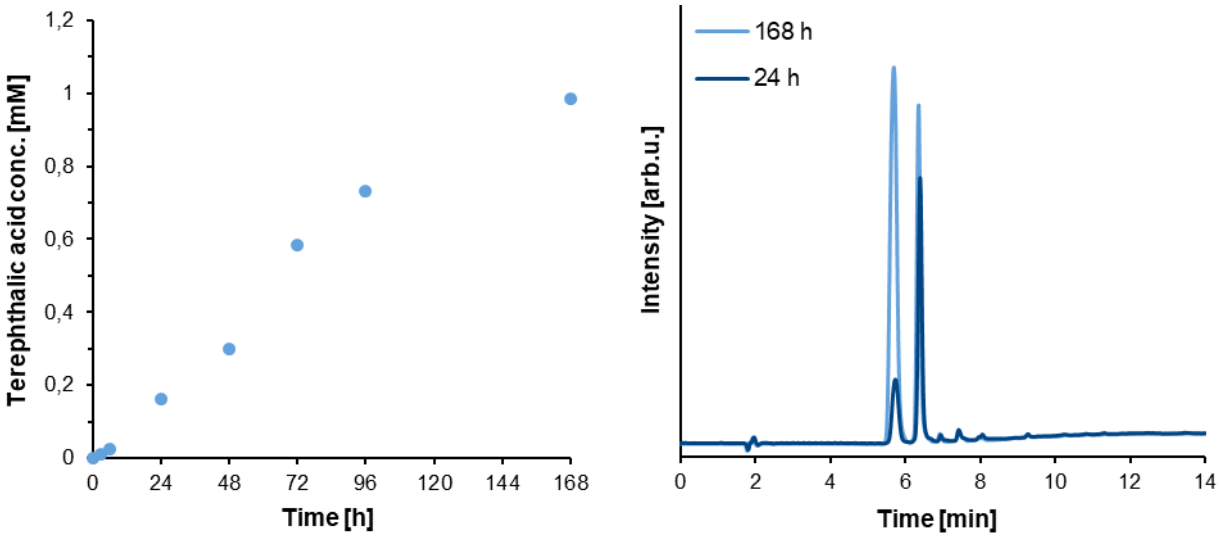

Figure 41: Results of hydrolysis of PET. Left: Terephthalic acid release during the degradation. Right: Chromatogram of supernatant after 24 and 168 hours. Two large peaks of TA (5.8 min) and MHET (6.3 min) are visible. Only small amounts of BHET (7.1 min) were released.

Enzymatic hydrolysis of PET proceeded slower than alkaline hydrolysis during pre-treatment experiments (chapter 4.2.1). After one-hour treatment with 1 M NaOH 1.7 mM terephthalic acid were released. The same amount was achieved after one day with cutinase.

FTIR analysis of PET resulted in identical spectra of the samples before and after the hydrolysis (Figure 42). Since the characteristic ratios did not differ significantly, a change in crystallinity could be excluded (Table 5).

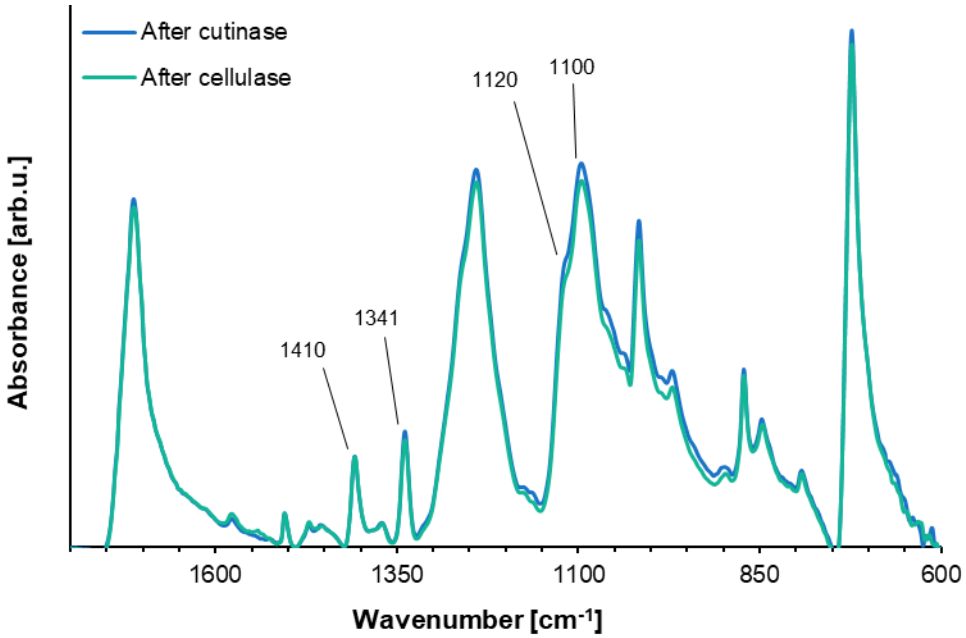

Figure 42: FTIR spectra of PET samples before and after cutinase treatment.

Table 5: Characteristic ratios for the determination of PET crystallinity via FTIR.

Sample	I 1120 / I 1100	A 1341 / A 1410
After cellulase	0.715	0.737
After cutinase	0.725	0.782

4.5 Ethanol fermentation

4.5.1 Ethanol fermentation of glucose from hydrolysis

After 18 hours glucose was used up by *S. cerevisiae* and ethanol production reached its maximum. At this points the yeast changed its metabolism and used the produced ethanol as carbon source. After an initial lag phase the ethanol concentration decreased and the cell density started to rise further, even though growth on ethanol was slower compared to glucose (Figure 43). The overall ethanol yield was 0.45 g ethanol/g glucose, which is close to the maximal theoretical yield 0.5 g/g (G. Walker & Stewart, 2016).

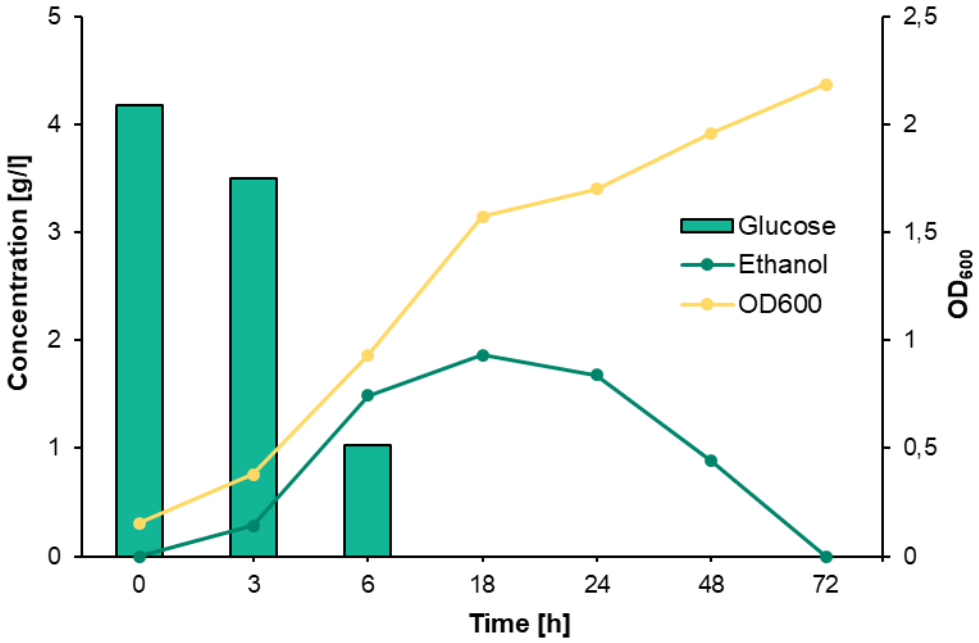

Figure 43: Ethanol production with *S. cerevisiae* from glucose obtained after enzymatic hydrolysis from cotton/PET blended textiles.

4.5.2 Comparing glucose sources for fermentation

The maximum ethanol yield was achieved during the fermentation with 0.2 % of commercial glucose, but overall all samples' yields were similar (Table 6). It seems that the medium containing hydrolysed cotton was available more readily to the microorganisms, as those had their maximum ethanol concentration after one day, whereas batches with commercial glucose did not produce ethanol that fast as shown in (Figure 44).

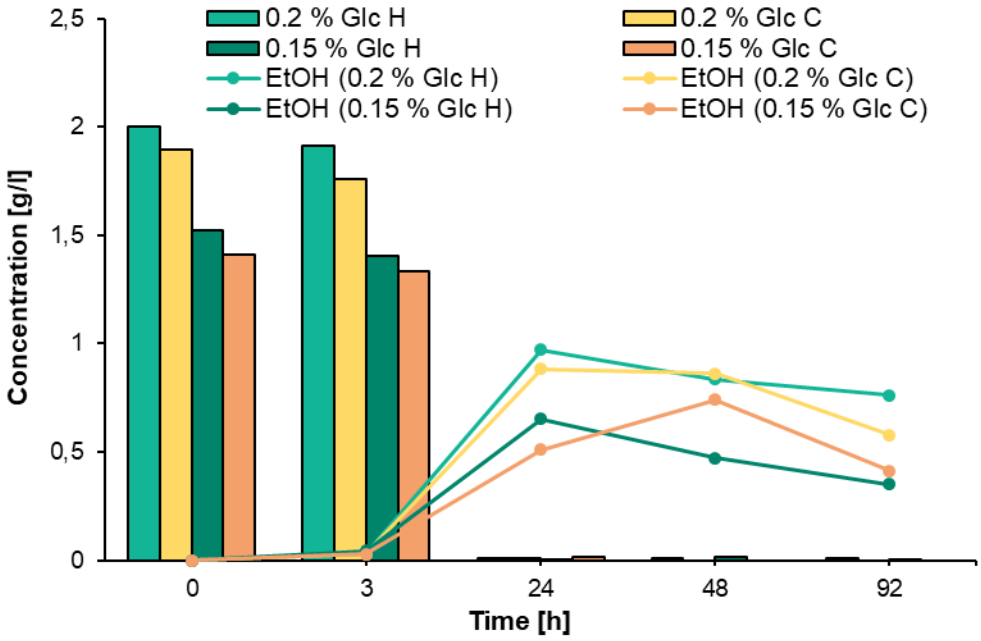

Figure 44: Comparing glucose from hydrolysis to commercial glucose as carbon source for fermentation.

Table 6: Ethanol yields for fermentation of commercial glucose and glucose from hydrolysis.

Sample	Yield [g EtOH/g glucose]
0.2 % Glc H	0.457
0.15 % Glc H	0.508
0.2 % Glc C	0.538
0.15 % Glc C	0.486

4.5.3 Comparison of aerobic and anaerobic fermentation

In all batches glucose was consumed within the first 18 hours. As demonstrated in the previous fermentation, under aerobic conditions *S. cerevisiae* used ethanol as carbon source once glucose was depleted. By contrast, anaerobic cultures did not metabolise ethanol, as oxygen is needed for this pathway. Therefore, ethanol concentration and biomass stayed constant (Figure 45).

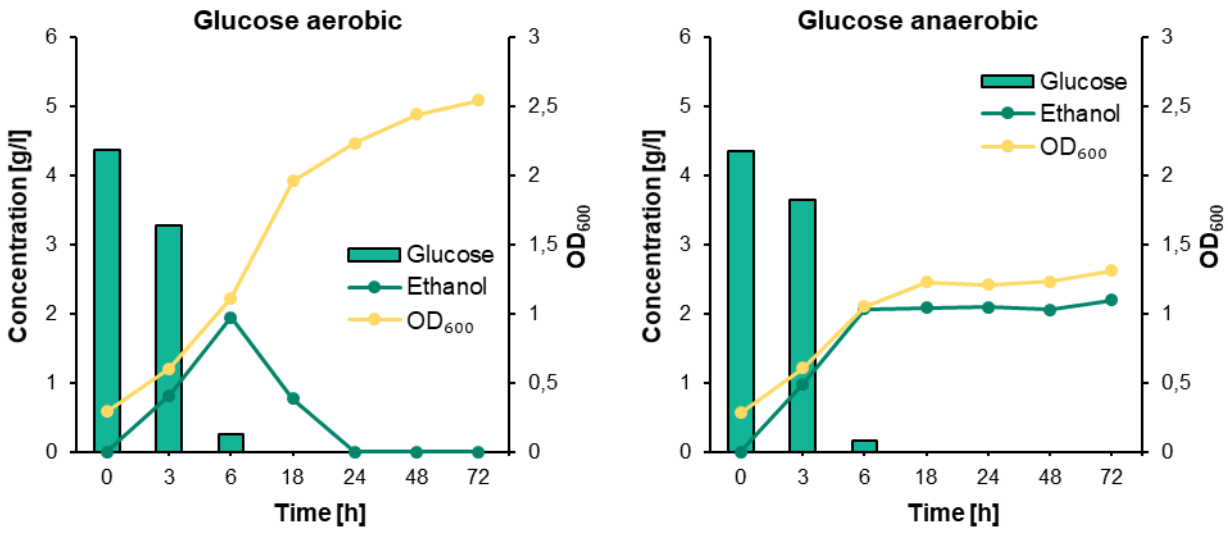

Figure 45: Comparison of anaerobic and aerobic ethanol fermentation with *S. cerevisiae*.

Cyrene decelerated the cell growth and resulted in lower ethanol yields in the aerobic culture. By contrast, ethanol concentrations further increased during anaerobic fermentation with Cyrene-addition, even after glucose was depleted (Figure 46). *S. cerevisiae* was able to metabolise Cyrene anaerobically, as shown by the HPLC results. The Cyrene peak at 30.5 minutes decreased over time, while a new unidentified peak, presumably associated to Cyrene-metabolites, appeared at 44 minutes (Figure 47). The yields of all four fermentations are listed in (Table 7).

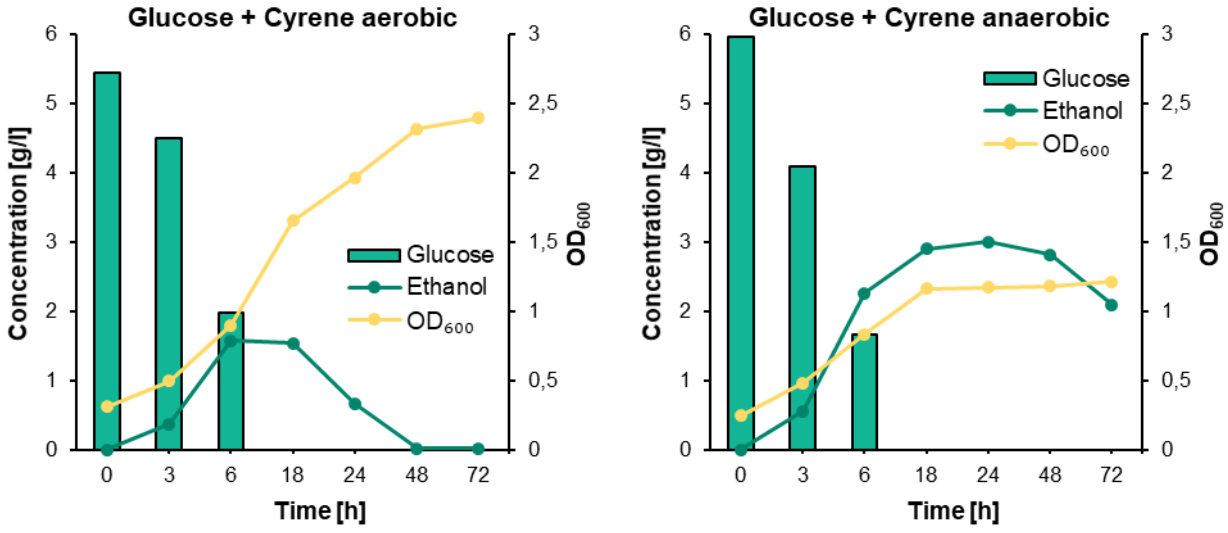

Figure 46: Influence of Cyrene on the aerobic and anaerobic ethanol fermentation with *S. cerevisiae.*

Table 7: Ethanol yields of anaerobic and aerobic fermentations with *S. cerevisiae*.

Sample	Yield [g EtOH/g glucose]
Glucose aerobic	0.446
Glucose anaerobic	0.506
Glucose + Cyrene aerobic	0.289
Glucose + Cyrene anaerobic	0.504

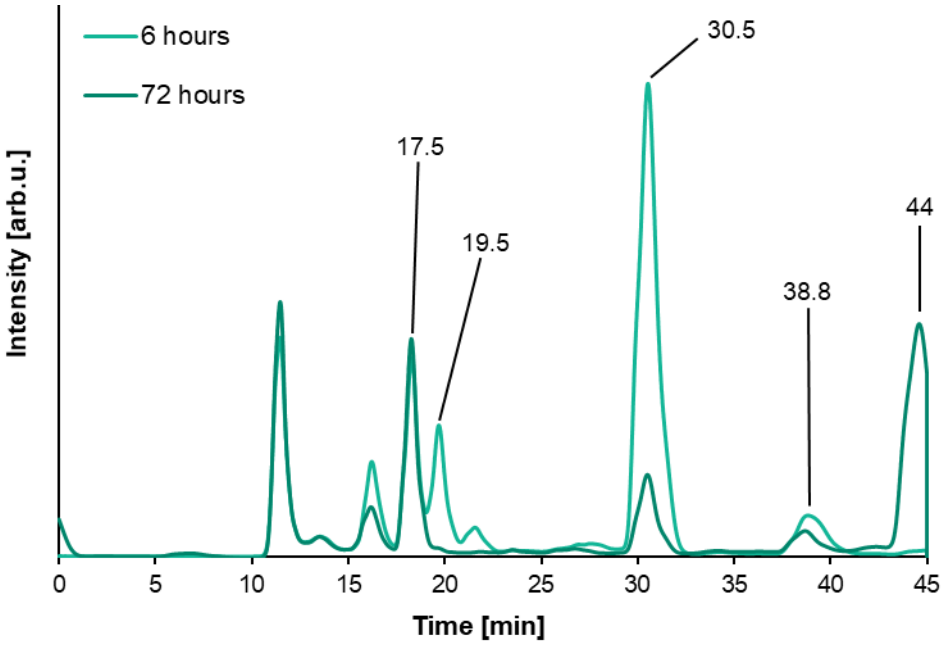

Figure 47: Chromatogram of fermentation broth from ethanol fermentation with *S. cerevisiae*. Retention times: Citric acid 17.5 min, glucose 19.5 min, Cyrene 30.5 min, ethanol 38.8 min, unidentified Cyrene metabolite 44 min.

4.6 Algae cultivation in cellulose hydrolysate

When no nitrogen source was supplemented, algae did not grow in the glucose solution derived from cotton hydrolysis. Upon addition of 1 g/l $NaNO_3$ OD_{600} slowly started to increase after 6 days (Figure 48). Since the pH was still low after cotton hydrolysis and no other nutrients were supplemented efficient growth was impossible.

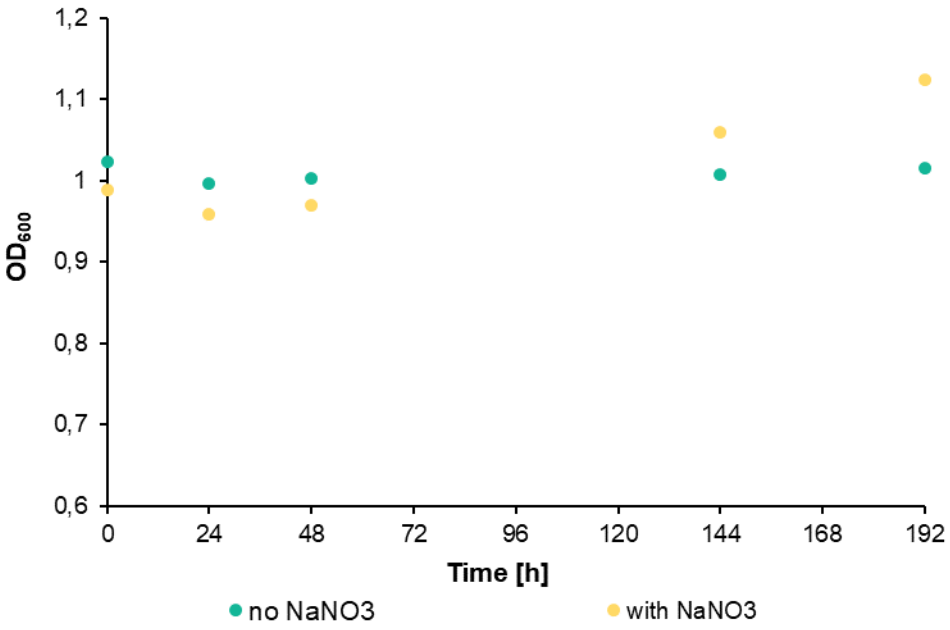

Figure 48: Algae growth on glucose derived from cotton hydrolysis.

4.7 *Trichoderma reesei* cultivation in cellulose hydrolysate

T. reesei was able to grow efficiently in yeast-peptone-medium supplemented with glucose from cotton hydrolysis. After three days the cultures grew highly dense, making the sample collection difficulty. Usually cellulose is needed to initiate the production of cellulases with *T. reesei*, which was not provided in this fermentation. Nevertheless, an increase of cellulytic activity was measured after 24 hours (227.92 U/ml). After 6 days the activity dropped to 62.46 U/ml, probably due to the presence of protease enzymes which were secreted by the fungus (Daranagama et al., 2019).

The SDS PAGE shows the increase in protein production in the first 4 days of fungi cultivation (Figure 49).

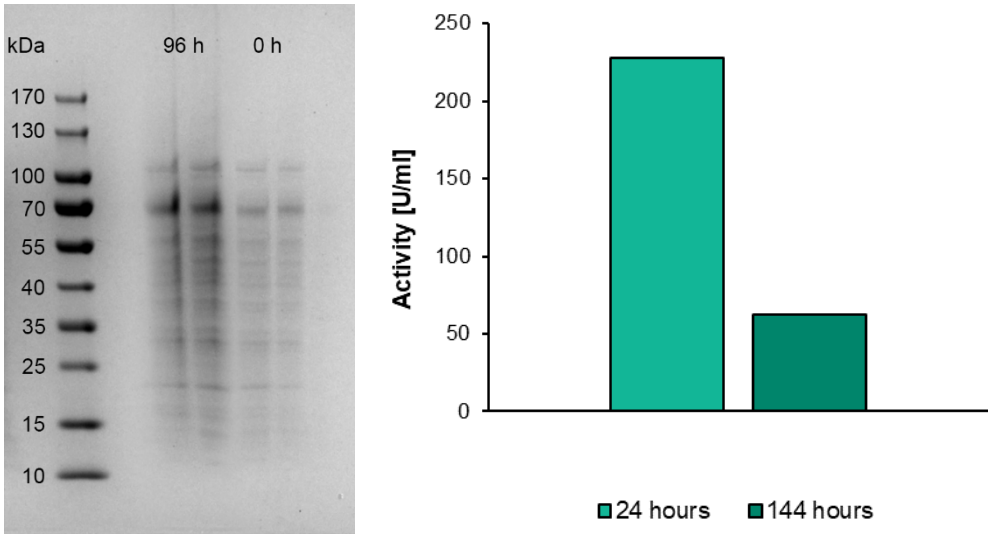

Figure 49: Characterisation of cellulases produced during *T. reesei* fermentation.

Glucose derived from cotton hydrolysis can be used to grow cellulase producing fungi. Upon initiation with cellulose the enzyme production could probably be increased.

5 Conclusion and outlook

The main goal of this thesis was to develop an enzyme-based process for the recovery of pure polyethylene terephthalate from blended cotton/PET-textiles. Cotton is rather resistant towards enzymatic hydrolysis due to its crystalline regions. As Zheng et al mentioned, effective pre-treatment methods could considerably facilitate the subsequent enzymatic hydrolysis (Zheng et al., 2009). Therefore, different ways to improve the enzymatic degradation of cotton were investigated by either by subjecting fibres to various pre-treatments or by changing parameters during the hydrolysis.

Reduction of textile size by mechanical processes like cutting and milling is a fundamental pre-treatment in order to increase the surface area of cotton providing more adhesion points for enzymes. A smaller sample size resulted in higher glucose concentrations and more weight loss, compared to more coarse or uncut samples.

During pre-treatment with high intensity ultrasound cellulose fibres were expected to fragment due to strong shear forces. During subsequent hydrolysis no improvements were observed compared to other hydrolyses were no such pre-treatment was conducted. Ultrasonic treatment is a complex subject and success deeply relies on choosing the right parameters. As multiple positive cases were reported, further studies in this direction could be performed (Luo et al., 2014).

Subjecting textiles to steam explosion visibly increased the volume of fibres suggesting that cellulose structures in general were loosened, providing enzymes access to cellulose chains. Alkaline pre-treatment using 0.5 M NaOH resulted in up to 1.8 % weight loss of cotton containing samples while the degradation of PET is in a negligible range. Only 0.04 mM terephthalic acid were measured after one hour of alkaline treatment with 0.5 M NaOH. The combination of these two pre-treatment methods lead to the highest glucose release of mixed fibres and the purest PET residues, which were produced during this thesis.

Increasing mass transfer is essential for the complete degradation of cotton. While mild shaking yielded higher glucose concentrations but fibres barely lost weight. Using a stirrer, on the other hand, resulted in a more exhaustive removal of cotton. The aprotic solvent Cyrene was added during the hydrolysis to interfere with cellulose's hydrogen bonding, increasing the internal surface area of cellulose fibres. Stability tests showed that the solvent decreases enzymatic activity by 50 % and leads to a further reduction of enzyme activity while the enzyme blank maintained a constant activity throughout the one-week incubation. Nevertheless, addition of 1 % Cyrene resulted in increased glucose

concentrations and overall more complete degradation of denim fibres during our experiments. Using higher concentrations of the solvent is not recommended as the negative effects became more prominent and results were generally worse than with just 1 % Cyrene.

Frequent exposure to ultrasound in an ultrasonic bath (42 kHz and 300 W) during the hydrolysis lead to 14 % reduced enzyme activity and worse degradation of cotton and can therefore not be suggested. As literature suggests that low frequency ultrasound could improve the performance of enzymatic degradation, further experiments could be conducted once another ultrasound source is available.

In another approach it was tried to improve the degradation by continuously removing glucose from the hydrolysis slurry via ultrafiltration. Substrate inhibition of enzymes was supposed to be prevented this way. Unfortunately, the initial reactor design let fibres escape the main chamber, resulting in blockage of the filtration system. Fibres which remained in the main chamber were of relatively pure PET even though the filtration system had to be tuned off after 2 days. Since first results were promising and a similar design has already proven its efficiency during hydrolysis of PET films (Barth et al., 2015) and lignocellulosic material (Andrić, Meyer, Jensen, & Dam-Johansen, 2010a), further research should be conducted in this direction.

After one week of hydrolysis cotton was not fully converted to glucose. Batches always had brown powder-like residues, which were visible once agitation was stopped and solids sedimented. These cotton-residues could easily be separated from PET via sedimentation and filtration during the washing process. The final washing process was an important step for the production of pure PET and is regarded as important as the pre-treatment. For faster purification of PET, a full enzymatic degradation of cotton might not be needed as long as good downstream processes are in use. PET could be separated from the slurry by mechanical means and the cotton residues could be further hydrolysed to generate more glucose.

PET residues were successfully recycled into PET pellets and spun into fibres by project partners. Despite having a brown colour from cotton impurities their mechanical properties were comparable to virgin PET. Mechanically recycled samples from the purest PET residues were not received in time but are expected being of white colour as FTIR analysis did not show any cotton impurities.

It was demonstrated that the accruing glucose can be used by microorganisms like *S. cerevisiae* for the production of ethanol or by *T. reesei*, which could be induced to produce cellulases, creating a self-sustaining enzyme cycle. During fermentation glucose

from hydrolysis performed the same as commercial glucose. Both resulted in yields around 0.5 g/g (g EtOH/g glucose). The presence of Cyrene did not interfere with the fermentation but, as it is a cellulose derived solvent, it could even be metabolised anaerobically by *S. cerevisiae*.

Enzymes could be recovered from the supernatant via ultrafiltration. However, the recovered enzyme solution only had 33 % of cellulase's initial enzyme's activity due to cellulases being stuck on the ultrafiltration membrane. If recovered enzymes were to be used in another hydrolysis, fresh cellulases would have to be supplemented as well to reach the desired activity.

Even though no definite process was developed, the concept of a true circular economy process was proven: cotton was degraded, PET was successfully recycled, and the accruing glucose was used for fermentation by different microorganisms for production of various value-added products

6 References

Adewuyi, Y. G., & Deshmane, V. G. (2015). Intensification of Enzymatic Hydrolysis of Cellulose Using High-Frequency Ultrasound: An Investigation of the Effects of Process Parameters on Glucose Yield. *Energy and Fuels*, *29*(8), 4998–5006. https://doi.org/10.1021/acs.energyfuels.5b00661

Alvira, P., Tomás-Pejó, E., Ballesteros, M., & Negro, M. J. (2010). Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. *Bioresource Technology*, *101*(13), 4851–4861. https://doi.org/10.1016/j.biortech.2009.11.093

Amed, I., Andersson, J., Berg, A., Drageset, M., Hedrich, S., & Kappelmark, S. (2017). The State of Fashion 2018. Retrieved October 22, 2019, from https://www.mckinsey.com/industries/retail/our-insights/renewed-optimism-for-the-fashion-industry

Andrić, P., Meyer, A. S., Jensen, P. A., & Dam-Johansen, K. (2010a). Effect and modeling of glucose inhibition and in situ glucose removal during enzymatic hydrolysis of pretreated wheat straw. *Applied Biochemistry and Biotechnology*, *160*(1), 280–297. https://doi.org/10.1007/s12010-008-8512-9

Andrić, P., Meyer, A. S., Jensen, P. A., & Dam-Johansen, K. (2010b). Reactor design for minimizing product inhibition during enzymatic lignocellulose hydrolysis. II. Quantification of inhibition and suitability of membrane reactors. *Biotechnology Advances*, *28*(3), 407–425. https://doi.org/10.1016/j.biotechadv.2010.02.005

Barth, M., Wei, R., Oeser, T., Then, J., Schmidt, J., Wohlgemuth, F., & Zimmermann, W. (2015). Enzymatic hydrolysis of polyethylene terephthalate films in an ultrafiltration membrane reactor. *Journal of Membrane Science*, *494*(029), 182–187. https://doi.org/10.1016/j.memsci.2015.07.030

Berg, J. M., Tymoczko, J. L., & Stryer, L. (2013). *Stryer Biochemie* (7th ed.). https://doi.org/https://doi.org/10.1007/978-3-8274-2989-6

Beton, A., Dias, D., Farrant, L., Gibon, T., Guern, Y. LE, Desaxce, M., … Boufateh, I. (2014). *Environmental Improvement Potential of Textiles (IMPRO Textiles), JRC Scientific and Technical Reports*. https://doi.org/10.2791/52624

Budtova, T., & Navard, P. (2015). Cellulose in NaOH–water based solvents: a review. *Springer Verlag*, *23*(1), 5–55. https://doi.org/10.1007/s10570-015-0779-8

Cotton: Science and technology. (2007). In S. Gordon & Y.-L. Hsieh (Eds.), *Woodhead Publishing Limited* (1st ed.). Woodhead Publishing Limited.

Cucchiella, F., D'Adamo, I., & Gastaldi, M. (2017). Sustainable waste management: Waste to energy plant as an alternative to landfill. *Energy Conversion and Management*, *131*, 18–31. https://doi.org/10.1016/j.enconman.2016.11.012

Daranagama, N. D., Shioya, K., Yuki, M., Sato, H., Ohtaki, Y., Suzuki, Y., … Ogasawara, W. (2019). Proteolytic analysis of Trichoderma reesei in celluase-inducing condition reveals a role for trichodermapepsin (TrAsP) in cellulase production. *Journal of Industrial Microbiology & Biotechnology*, *46*(6), 831–842. https://doi.org/10.1007/s10295-019-02155-9

De Silva, R., Wang, X., & Byrne, N. (2014). Recycling textiles: The use of ionic liquids in the separation of cotton polyester blends. *RSC Advances*, *4*(55), 29094–29098. https://doi.org/10.1039/c4ra04306e

Dimarogona, M., Topakas, E., & Christakopoulos, P. (2012). Cellulose degradation by

oxidative enzymes. *Computational and Structural Biotechnology Journal*. https://doi.org/10.5936/csbj.201209015

Divne, C., Ståhlberg, J., Reinikainen, T., Ruohonen, L., Knowles, J. K. C., Teeri, T. T., & Jones, T. A. (1994). The Three-Dimensional Crystal Structure of the Catalytic Core of Cellobiohydrolase I from Trichoderma reesei. *Advancement Of Science*, *265*(5171), 524–528. https://doi.org/10.1126/science.8036495

Donelli, I., Taddei, P., Smet, P. F., Poelman, D., Nierstrasz, V. A., & Freddi, G. (2009). Enzymatic surface modification and functionalization of PET: A water contact angle, FTIR, and fluorescence spectroscopy study. *Biotechnology and Bioengineering*, *103*(5), 845–856. https://doi.org/10.1002/bit.22316

ecoplus. Niederösterreichs Wirtschaftsagentur GmbH. (2017). Tex2Mat: Entwicklung neuer Aufbereitungs-Methoden und Prozesse zum Recycling von Textilabfällen multi-materialer Zusammensetzung.

Eerhart, A. J. J. E., Faaij, A. P. C., & Patel, M. K. (2012). Replacing fossil based PET with biobased PEF; Process analysis, energy and GHG balance. *Energy and Environmental Science*, *5*(4), 6407–6422. https://doi.org/10.1039/c2ee02480b

European Commission. (2019). Report on the implementation of the Circular Economy Action Plan, {SWD(2019) 90 final}. Retrieved October 22, 2019, from https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52019DC0190&from=EN

European Parliament, & European Council. (2008). Directive 2008/98/EC of the European Parliament and of the Council of 19 November 2008 on waste and repealing certain Directives (Text with EEA relevance). Retrieved October 22, 2019, from http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32008L0098&from=EN

Fink, H.-P., Weigel, P., Purz, H. J., & Ganster, J. (2001). Structure formation of regenerated cellulose materials from NMMO-solutions. *Progress in Polymer Science*, *26*(9), 1473–1524. https://doi.org/https://doi.org/10.1016/S0079-6700(01)00025-9

Frieden, E., & Walter, C. (1963). Prevalence and Significance of the Product Inhibition of Enzymes. *Nature*, *198*(4883), 834–837. https://doi.org/10.1038/198834a0

Gray, S. (2017). *Mapping clothing impacts in Europe: the environmental cost* (p. 41). p. 41. Retrieved from http://www.ecap.eu.com/wp-content/uploads/2018/07/Mapping-clothing-impacts-in-Europe.pdf

Gubbels, E., Heitz, T., Yamamoto, M., Chilekar, V., Zarbakhsh, S., Gepraegs, M., … Kaminsky, W. (2018). Polyesters. *Ullmann's Encyclopedia of Industrial Chemistry*. https://doi.org/https://doi.org/10.1002/14356007.a21_227.pub2

Hawley, J. M. (2014). Textile Recycling. *Handbook of Recycling: State-of-the-Art for Practitioners, Analysts, and Scientists*, 211–217. https://doi.org/10.1016/B978-0-12-396459-5.00015-5

Henriksson, H., Ståhlberg, J., Isaksson, R., & Pettersson, G. (1996). The active sites of cellulases are involved in chiral recognition: A comparison of cellobiohydrolase 1 and endoglucanase 1. *FEBS Letters*, *390*(3), 339–344. https://doi.org/10.1016/0014-5793(96)00685-0

Imai, T., Boisset, C., Samejima, M., Igarashi, K., & Sugiyama, J. (1998). Unidirectional processive action of cellobiohydrolase Cel7A on Valonia cellulose microcrystals. *FEBS Letters*, *432*(3), 113–116. https://doi.org/10.1016/S0014-5793(98)00845-X

Jeihanipour, A., Karimi, K., Niklasson, C., & Taherzadeh, M. J. (2010). A novel process for ethanol or biogas production from cellulose in blended-fibers waste textiles. *Waste Management*, *30*(12), 2504–2509. https://doi.org/10.1016/j.wasman.2010.06.026

Kalliala, E. M., & Nousiainen, P. (1999). Environmental profile of cotton and polyester-cotton

fabrics. *Autex Research Journal*, *1*(1), 8–20.

Kleman-Leyer, K., Agosin, E., Conner, A. H., & Kirk, T. K. (1992). Changes in molecular size distribution of cellulose during attack by white rot and brown rot fungi. *Applied and Environmental Microbiology*, *58*(4), 1266–1270.

Kosmidis, V. A., Achilias, D. S., & Karayannidis, G. P. (2001). Poly(ethylene terephthalate) Recycling and Recovery of Pure Terephthalic Acid. Kinetics of a Phase Transfer Catalyzed Alkaline Hydrolysis. *Macromolecular Materials and Engineering*, *286*(10), 640–647. https://doi.org/10.1002/1439-2054(20011001)286:10<640::AID-MAME640>3.0.CO;2-1

Lee, Y. -H, & Fan, L. T. (1982). Kinetic studies of enzymatic hydrolysis of insoluble cellulose: Analysis of the initial rates. *Biotechnology and Bioengineering*, *24*(11), 2383–2406. https://doi.org/10.1002/bit.260241107

Lehtiö, J., Sugiyama, J., Gustavsson, M., Fransson, L., Linder, M., & Teeri, T. T. (2003). The binding specificity and affinity determinants of family 1 and family 3 cellulose binding modules. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(2), 484–489. https://doi.org/10.1073/pnas.212651999

Lin, Y., & Tanaka, S. (2006). Ethanol fermentation from biomass resources: Current state and prospects. *Applied Microbiology and Biotechnology*, *69*(6), 627–642. https://doi.org/10.1007/s00253-005-0229-x

Lindman, B., Karlström, G., & Stigsson, L. (2010). On the mechanism of dissolution of cellulose. *Journal of Molecular Liquids*, *156*(1), 76–81. https://doi.org/10.1016/j.molliq.2010.04.016

Luo, J., Fang, Z., & Smith, R. L. (2014). Ultrasound-enhanced conversion of biomass to biofuels. *Progress in Energy and Combustion Science*, *41*(1), 56–93. https://doi.org/10.1016/j.pecs.2013.11.001

Lynd, L. R., Weimer, P. J., Zyl, W. H. van S., & Pretorius, I. (2002). Microbial Cellulose Utilization: Fundamentals and Biotechnology. *Microbiology and Molecular Biology Reviews*, *66*(3), 506–577. https://doi.org/10.1128/MMBR.66.3.506-577.2002

MacArthur, E. (2017). A new textiles economy: Redesigning fashion's future. *Ellen MacArthur Foundation*, pp. 1–150. Retrieved from https://www.ellenmacarthurfoundation.org/publications/a-new-textiles-economy-redesigning-fashions-future

Mather, R. R., & Wardman, R. H. (2015). *The Chemistry of Textile Fibres* (2nd ed.). The Royal Society of Chemistry.

Motte, H. De, & Palme, A. (2018). *The development of the Blend Re:wind process*.

Nguyen, T. T. T., & Le, V. V. M. (2013). Effects of ultrasound on cellulolytic activity of cellulase complex. *International Food Research Journal*, *20*(2), 557–563.

Ouchi, A., Toida, T., Kumaresan, S., Ando, W., & Kato, J. (2010). A new methodology to recycle polyester from fabric blends with cellulose. *Cellulose*, *17*(1), 215–222. https://doi.org/https://doi.org/10.1007/s10570-009-9358-1

Payne, A. (2015). Open- and closed-loop recycling of textile and apparel products. In *Handbook of Life Cycle Assessment (LCA) of Textiles and Clothing*. https://doi.org/10.1016/b978-0-08-100169-1.00006-x

Pellis, A., Herrero Acero, E., Gardossi, L., Ferrario, V., & Guebitz, G. M. (2016). Renewable building blocks for sustainable polyesters: new biotechnological routes for greener plastics. *Polymer International*, *65*(8), 861–871. https://doi.org/10.1002/pi.5087

Peterson, A., Palme, A., Brelid, H., de la Motte, H., & Theliander, H. (2017). Development

of an efficient route for combined recycling of PET and cotton from mixed fabrics. *Textiles and Clothing Sustainability*, *3*(1). https://doi.org/10.1186/s40689-017-0026-9

Promega Corporation. (2009). Calculating Nucleic Acid or Protein Concentration Using the GloMax® Multi+ Microplate Instrument. Retrieved October 22, 2019, from https://www.promega.com/-/media/files/resources/application-notes/pathlength/calculating-nucleic-acid-or-protein-concentration-using-the-glomax-multi-microplate-instrument.pdf?la=en

Quartinello, F., Vecchiato, S., Weinberger, S., Kremenser, K., Skopek, L., Pellis, A., & Guebitz, G. M. (2018). Highly selective enzymatic recovery of building blocks fromwool-cotton-polyester textile waste blends. *Polymers*, *10*(10). https://doi.org/10.3390/polym10101107

Reichel, A., Mortensen, L. F., Asquith, M., & Bogdanovic, J. (2014). Environmental indicator report 2014. *20 Oct 2014*, 1–95. https://doi.org/10.2800/22394

Remy, N., Speelman, E., & Swartz, S. (2016). Style that's sustainable : A new fast-fashion formula. Retrieved October 22, 2019, from https://www.mckinsey.com/business-functions/sustainability/our-insights/style-thats-sustainable-a-new-fast-fashion-formula

Rosatella, A. A., Simeonov, S. P., Frade, R. F. M., & Afonso, C. A. M. (2011). 5-Hydroxymethylfurfural (HMF) as a building block platform: Biological properties, synthesis and synthetic applications. *Green Chemistry*, *13*(4), 754–793. https://doi.org/10.1039/c0gc00401d

Sandgren, M., Shaw, A., Ropp, T. H., Wu, S., Bott, R., Cameron, A. D., … Jones, T. A. (2001). The X-ray crystal structure of the Trichoderma reesei family 12 endoglucanase 3, Cel12A, at 1.9 Å resolution. *Journal of Molecular Biology*, *308*(2), 295–310. https://doi.org/10.1006/jmbi.2001.4583

Sattler, H., & Schweizer, M. (2011). Fibers, 5. Polyester Fibers. *Ullmann's Encyclopedia of Industrial Chemistry*. https://doi.org/https://doi.org/10.1002/14356007.o10_o01

Schultz, T. P., Blermann, C. J., & Mcginnis, G. D. (1983). Steam Explosion of Mixed Hardwood Chips as a Biomass Pretreatment. *Industrial and Engineering Chemistry Product Research and Development*, *22*(2), 344–348. https://doi.org/10.1021/i300010a034

Seidl, V., Seibel, C., Kubicek, C. P., & Schmoll, M. (2009). Sexual development in the industrial workhorse Trichoderma reesei. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(33), 13909–13914. https://doi.org/10.1073/pnas.0904936106

Shen, L. (2011). *Bio-based and recycled polymers for cleaner production* (University Utrecht). Retrieved from https://dspace.library.uu.nl/handle/1874/202398

Sherwood, J., De Bruyn, M., Constantinou, A., Moity, L., McElroy, C. R., Farmer, T. J., … Clark, J. H. (2014). Dihydrolevoglucosenone (Cyrene) as a bio-based alternative for dipolar aprotic solvents. *Chemical Communications*, *50*(68), 9650–9652. https://doi.org/10.1039/c4cc04133j

Statistical Office of the European Communities. (2019a). Circular material Rate in EU. Retrieved September 21, 2019, from https://ec.europa.eu/eurostat/tgm/table.do?tab=table&init=1&language=en&pcode=cei_srm030&plugin=1

Statistical Office of the European Communities. (2019b). Household expenses on clothing. Retrieved September 21, 2019, from http://appsso.eurostat.ec.europa.eu/nui/show.do?query=BOOKMARK_DS-423035_QID_-76AC1A1D_UID_-

3F171EB0&layout=COICOP,B,X,0;TIME,C,Y,0;UNIT,L,Z,0;GEO,L,Z,1;INDICATORS,C,Z,2;&zSelection=DS-423035INDICATORS,OBS_FLAG;DS-423035GEO,EU28;DS-423035UNIT,PC_TOT;&rank

Statistical Office of the European Communities. (2019c). Turnover of Clothing Industry in the EU. Retrieved September 21, 2019, from http://appsso.eurostat.ec.europa.eu/nui/show.do?query=BOOKMARK_DS-120933_QID_7B649D27_UID_-3F171EB0&layout=TIME,C,X,0;NACE_R2,L,Y,0;GEO,L,Z,0;INDIC_SB,L,Z,1;INDICATORS,C,Z,2;&zSelection=DS-120933GEO,EU28;DS-120933INDICATORS,OBS_FLAG;DS-120933INDIC_SB,V121

Sulaiman, A. Z., Ajit, A., & Chisti, Y. (2013). Ultrasound mediated enzymatic hydrolysis of cellulose and carboxymethyl cellulose. *Biotechnology Progress*, *29*(6), 1448–1457. https://doi.org/10.1002/btpr.1786

Swatloski, R. P., Spear, S. K., Holbrey, J. D., & Rogers, R. D. (2002). Dissolution of cellose with ionic liquids. *Journal of the American Chemical Society*, *124*(18), 4974–4975. https://doi.org/10.1021/ja025790m

Szabó, O. E., & Csiszár, E. (2013). The effect of low-frequency ultrasound on the activity and efficiency of a commercial cellulase enzyme. *Carbohydrate Polymers*, *98*(2), 1483–1489. https://doi.org/10.1016/j.carbpol.2013.08.017

Viet, D., Beck-Candanedo, S., & Gray, D. G. (2007). Dispersion of cellulose nanocrystals in polar organic solvents. *Cellulose*, *14*(2), 109–113. https://doi.org/10.1007/s10570-006-9093-9

Vinzant, T. B., Adney, W. S., Decker, S. R., Baker, J. O., Kinter, M. T., Sherman, N. E., … Himmel, M. E. (2001). Fingerprinting Trichoderma reesei hydrolases in a commercial cellulase preparation. *Applied Biochemistry and Biotechnology*, *91*(1–9), 99–107. https://doi.org/10.1385/ABAB:91-93:1-9:99

Walker, G. M. (1998). *Yeast Physiology and Bioterchnology* (1st ed., Vol. 1). John Wiley & Sons Ltd.

Walker, G., & Stewart, G. (2016). Saccharomyces cerevisiae in the Production of Fermented Beverages. *Beverages*, *2*(4), 30. https://doi.org/10.3390/beverages2040030

Walls, D. J. (1991). Application of ATR-IR to the Analysis of Surface Structure and Orientation in Uniaxially Drawn Poly(ethyleneterephthalate). *Applied Spectroscopy*, *45*(7), 1193–1198. https://doi.org/10.1366/0003702914336147

Watson, D., Aare, A. K., Trzepacz, S., & Peterse, C. D. (2018). Used Textile Collection in European Cities. Study commissioned by Rijkswaterstaat under the European Clothing Action Plan (ECAP). Retrieved November 22, 2019, from http://www.ecap.eu.com/wp-content/uploads/2018/07/ECAP-Textile-collection-in-European-cities_full-report_with-summary.pdf

Xiao, Z., Zhang, X., Gregg, D. J., & Saddler, J. N. (2004). Effects of sugar inhibition on cellulases and β-glucosidase during enzymatic hydrolysis of softwood substrates. *Applied Biochemistry and Biotechnology - Part A Enzyme Engineering and Biotechnology*, *115*(1–3), 1115–1126. https://doi.org/10.1385/ABAB:115:1-3:1115

Yim, H., Haselbeck, R., Niu, W., Pujol-Baxley, C., Burgard, A., Boldt, J., … Van Dien, S. (2011). Metabolic engineering of Escherichia coli for direct production of 1,4-butanediol. *Nature Chemical Biology*, *7*, 445. Retrieved from https://doi.org/10.1038/nchembio.580

Zhao, Y., Liu, X., Wang, J., & Zhang, S. (2013). Insight into the cosolvent effect of cellulose dissolution in imidazolium-based ionic liquid systems. *Journal of Physical Chemistry*

B, *117*(30), 9042–9049. https://doi.org/10.1021/jp4038039

Zheng, Y., Pan, Z., & Zhang, R. (2009). Overview of biomass pretreatment for cellulosic ethanol production. *International Journal of Agricultural and Biological Engineering*, *2*(3), 51–68. https://doi.org/10.3965/j.issn.1934-6344.2009.03.051-068