ABSTRACT

EGAN, JEAN MARIE. Enzymatic Degradation of Textile Waste as a Pathway for Textile Circularity. (Under the direction of Dr. Sonja Salmon).

Simulated and commercial cellulose-containing textile waste substrates were degraded using a multicomponent cellulase enzyme formulation. After 19 hours of enzymatic treatment at a sufficient enzyme dose with an aggressive level of mechanical agitation, a bleached white cotton fabric was converted from controlled fabric swatches into a fiber slurry, consisting of recalcitrant fibers and soluble sugars. Isolated addition of chemical additives including a monofunctional or bifunctional reactive dye or a durable press crosslinking agent inhibited enzymatic hydrolysis. A bifunctional reactive dye had a more significant impact on fabric hydrolysis than a monofunctional dye because of fiber crosslinking. With sufficient time and enzyme dose, the reactive dyed fabrics were fully converted into a slurry. A durable press finished fabric showed a substantial impact on degradation, which was decreased by 90% from the bleached white cotton fabric treated under the same conditions. A chemoenzymatic pathway was developed with acid and alkali pretreatment steps that allowed for hydrolysis of the chemical crosslinks before enzymatic hydrolysis of the fabric. After chemical pretreatment and enzymatic treatment, a durable press finished fabric was fully degraded into a slurry.

Enzymatic hydrolysis of a 50/50 PET/cotton blended fabric fully removed cotton from the blend, leaving behind a cleaned polyester fabric despite the synthetic fibers acting as a physical block to cotton degradation. Other blended fabrics that had added chemical obstacles were tested. Cotton from a dyed 55/45 modacrylic/cotton commercial fabric was successfully removed through enzymatic treatment. A crosslinked and dyed 55/45 modacrylic/cotton fabric was treated under the same chemoenzymatic conditions as the durable press 100% cotton fabric, removing about 80% of the cotton after 38 hours of enzymatic treatment.

Degradation products including the soluble products from bleached white cotton, small solids (<2mm fibers) from several fabrics, and large solids from blended fabrics were characterized to understand their potential for reuse in recycled or circular applications. Through LC-CAD analysis, it was determined that the soluble sugars generated through the methodology were primarily glucose, which is a useful feedstock for fermentation. Fiber quality analysis [FQA] of fine fibers showed an inverse relationship between fabric degradation and fiber length. Further, a vast majority of fibers released during this process were less than 100μm in length. Small solids were also shown to have higher crystallinity index than their original fabric crystallinity through X-ray diffraction [XRD], demonstrating that enzymes preferentially attack amorphous fiber regions first. The short length and high crystallinity of the recalcitrant fiber product are both potentially beneficial attributes for recycling applications.

Composition and thermal properties of recovered large PET solids were analyzed in comparison to several reference fabrics. Fourier-transform infrared [FTIR] analysis showed that the cleaned PET sample shared the same characteristic peaks as a PET reference and did not share any peaks with a cotton reference. Thermogravimetric analysis [TGA] similarly showed that the cleaned PET had an unchanged degradation profile from the reference PET sample. Differential scanning calorimetry [DSC] showed unchanged onset and peak melting temperatures between a PET reference and the enzymatically cleaned PET sample, though there appeared to be some differences in quality between the two PET samples. However, PET quality did not differ from its original 50/50 PET/cotton fabric. This, in conjunction with the other analytical techniques employed, led to the conclusion that the enzymatic treatment did not cause any noticeable damage to the PET fibers, meaning they should be suitable for use in mechanical or chemical recycling pathways.

Enzymatic Degradation of Textile Waste as a Pathway for Textile Circularity

by
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BIOGRAPHY

Jeannie Egan was born in Greensboro, North Carolina and began studying at the Wilson College of Textiles in 2017. She joined the Textile Biocatalysis Research Group under Dr. Sonja Salmon in 2019, where she found her interest in textile end-of-life and circularity. After completing her degree in Polymer and Color Chemistry in 2021, she started pursuing a Textile Chemistry master's degree at the college, focusing on textile recycling through enzymatic hydrolysis under Dr. Salmon. This work has given her many outreach opportunities, including speaking at several conferences, most recently WasteExpo in New Orleans and ITMA in Milan in 2023.

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1. INTRODUCTION

Among many other prevalent concerns of textile sustainability, textile materials do not disappear when they are thrown away. With emphasis on textile sustainability in product development growing due to plastics legislation and consumer recognition of environmental issues with textiles, there has been an increase in awareness of the textile waste accumulation issue at both the industry and consumer level. Textile waste not only accounts for post-consumer waste but also includes production waste. In cotton yarn spinning alone, it is estimated that between 13-30% of every bale of cotton is discarded because of quality issues [2]. Other pre-consumer waste examples include selvage from woven fabrics (or other cut-and-sew waste), fabrics with construction defects because of machine failure, and fabrics with misprinted patterns.

Of the over 2 billion tons of solid waste generated in 2015 [3], textiles made up between 3-5%, estimated between 65-92 million tons [4, 5]. These estimates are summarized and approximated by the Ellen MacArthur foundation as being one garbage truck full of textiles disposed of in landfills every second [6]. In 2018, the US disposed of 10-11.5 million tons of textile waste in landfills [7, 8]. Textile data is not as regularly collected as other categories like food waste and plastic waste, so there is some debate over the estimates presented [8].

Because textiles are built to be durable, they also characteristically are environmentally persistent, meaning they stay intact or otherwise do not degrade for extended periods of time [9], leading to a significant amount of solid organic waste accumulation. It has been estimated that 28-60% of textiles that are disposed of are suitable for immediate reuse [10, 11], though facilitation of this immediate reuse is only now emerging. If no recycling or alternative disposal methods are implemented by 2040, an estimated 4.5 gigatons of textile solid waste will have accumulated on the planet, whether still in fabric form or otherwise partially degraded into microfibers [12].

Textiles currently exist in a linear supply and disposal pathway. Most new textiles are generated using virgin raw materials like cotton or petrochemicals for synthetic polymers. Once textiles are used by the consumer, they are typically thrown away and disposed of in landfills, where they spend the extended post-consumer segment of their life cycle. Because of newfound consumer demand for sustainable clothing as well as corporate-level sustainable emphasis in some textile design spaces, there is a push to move towards a more circular textile-to-textile supply chain model, which has generated a lot of research and innovation in the field. A circular production model requires intention, where products must be designed in coordination with research efforts for disassembly and reuse, such that the lifespan of a product can be extended as much as realistically possible [3].

Since polyethylene terephthalate [PET] and cellulose make up about 88% of global textile fiber production [13], most recycling technologies target either one of these fiber types or recycling blends containing both fibers. In the case of PET and cotton, the highly variable chemo-physical properties of the two fibers can be taken advantage of to separate them into individually recyclable components [14, 15]. Some fiber separation efforts have investigated dissolving of the PET component in a blend, such that the PET is broken down to its monomers which can be recovered for synthesis into new PET fibers and the residual cotton solids can be recovered and recycled [16]. Other efforts have investigated dissolution of cotton from the blend using high concentration sulfuric acid [17], phosphoric acid [18], or hydrochloric acid [19]. These methods destroy the cotton portion, which is then disposed of, leaving a solid PET fraction that could be recycled. There are some concerns about the quality of the recovered PET after acid treatment, which are known to damage the chemical structure through hydrolysis [20]. Further, those strong solvents can be hard to recycle [21], also requiring significant processing before disposal. As an alternative to these

acids, cellulase enzymes can perform selective degradation on the cotton or other cellulosic portion of a blend, leaving an undamaged synthetic portion behind [22].

A major objective of this work was to further investigate enzymatic hydrolysis of cellulosic textiles, primarily cotton, and characterizing degradation byproducts to support the validity of the hydrolysis process as a recycling pathway for cotton textiles. In order to study this, cotton fabrics were prepared to simulate realistic textile waste substrates, including a 100% bleached cotton jersey fabric and isolated addition of chemical obstacles like a monofunctional or bifunctional reactive dye or a durable press crosslinking finish. A 50/50 polyester/cotton blended fabric was also tested to determine the efficiency and efficacy of enzymatic separation of blended fibers. Findings from these tests were supplemented by viscose fabric hydrolysis and testing commercially available textile samples that combined multiple of the identified obstacles.

Literature on enzymatic hydrolysis is varied in its hydrolysis methodology in terms of machinery and sample preparation, so preliminary studies were conducted to create a set of standard degradation conditions in the chosen 2mag dry bath mixer used for degradation experiments. The 2mag was chosen because of its ability to process up to 15 samples with controlled insulated heating and individual magnetic stirring for each sample. Process parameters like controlled fabric piece size, sample mass, liquor ratio, and reaction time were all individually studied and optimized for the baseline bleached cotton fabric. Further adjustments to the standard conditions were made for other fabrics as needed. Most testing was carried out using a multicomponent cellulase mixture, Cellic® CTec2, though some experiments used experimental monocomponent cellulase formulations. Cellulase enzyme activity was quantified by reducing sugar [RS] assay and reported in filter paper units [FPU/mL of product]. Degradation was quantified through gravimetric analysis of the solid degradation products: large solids which were

greater than 2mm fabric pieces and small solids which were less than 2mm in any dimension. Any unaccounted-for mass from the original sample, representing the fabric portion fully converted to soluble components, was quantified as weight loss.

Several methods were used to characterize degradation products which included recovered synthetic solids for blended fabrics and recovered recalcitrant cotton solids and soluble sugar components from all tested fabrics. PET solids recovered after enzymatic removal of the cotton component existed in a lightweight fabric form, which was characterized using Fourier transform infrared [FTIR], thermogravimetric [TGA], scanning electron microscopy [SEM], and differential scanning calorimetry [DSC] analyses. Recalcitrant cotton solids, or cotton fiber fragments, were characterized by X-ray diffraction [XRD] and fiber quality analysis [FQA]. Soluble sugar components were identified and quantified using liquid chromatography with charged aerosol detector [LC-CAD]. Some of this characterization data was further analyzed using SAS JMP Pro 17 software for statistical analysis or Origin 2023b for raw data interpretation.

2. LITERATURE REVIEW

2.1 Enzymatic Hydrolysis

2.1.1 Cellulase Enzymes

Cellulase enzymes refer to a category of highly sophisticated proteins that carry out specific degradation reactions on cellulosic substrates (Figure 2.1.1.1). There is not one singular cellulase protein that will degrade cellulose from polymer to glucose, so nature produces proteins that carry out different catalytic functions as a means to accomplish full conversion to glucose [23]. The three major cellulase categories that are relevant are endoglucanases (EG), cellobiohydrolases (CBH), and beta-glucosidases (BG).

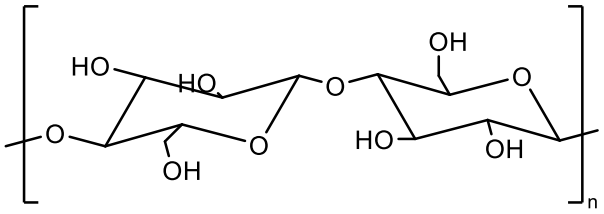
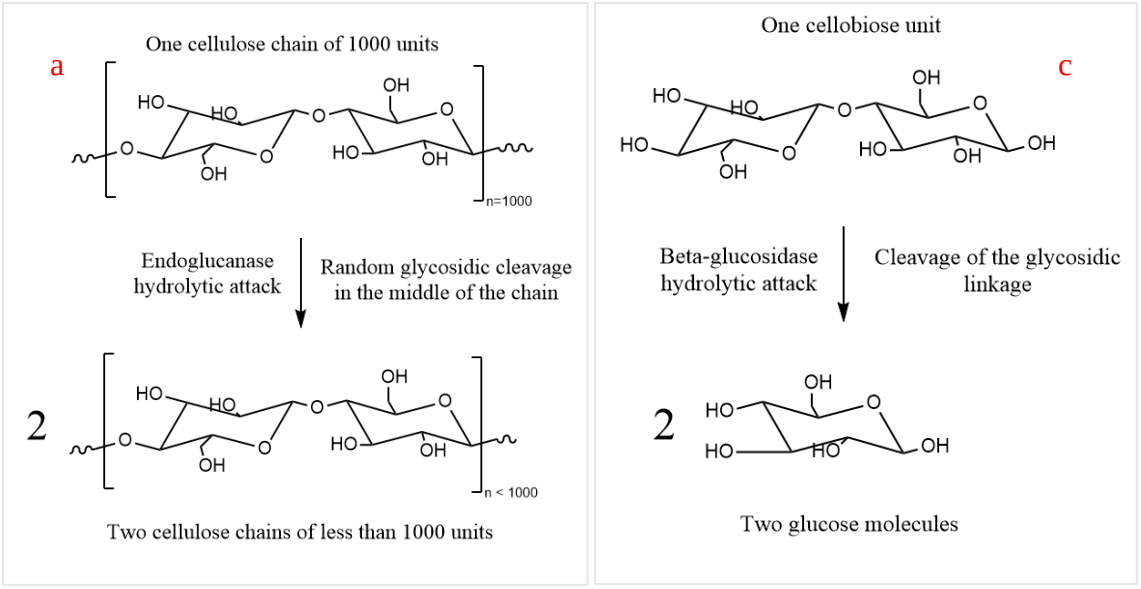


Figure 2.1.1.1. Cellulose polymer repeat unit.

Endoglucanases randomly cleave beta-1,4-glycosidic bonds between glucan units along the cellulose backbone (Figure 2.1.1.2a), having higher affinity for amorphous fiber regions [24]. EGs are the portion of a cellulase mixture that carry out bulk polymer fragmentation [22, 25], and after initial endo-type attack, will leave a more crystalline substrate [24]. If allowed to react long enough beyond gross fragmentation, EGs can also release cellobiose [23], mimicking an exo-type reaction. Cellobiohydrolases intentionally carry out this exo-hydrolysis of cellulose chains (Figure 2.1.1.2b), being the cellulase component mainly responsible for releasing cellobiose by attacking the end of chains [25]. For both EG and CBH, cellobiose is a known inhibitor to prolonged hydrolysis [26]. Beta-glucosidases are responsible for the final conversion into glucose (Figure 2.1.1.2c), hydrolyzing cellobiose mainly and sometimes other short oligosaccharides [23].



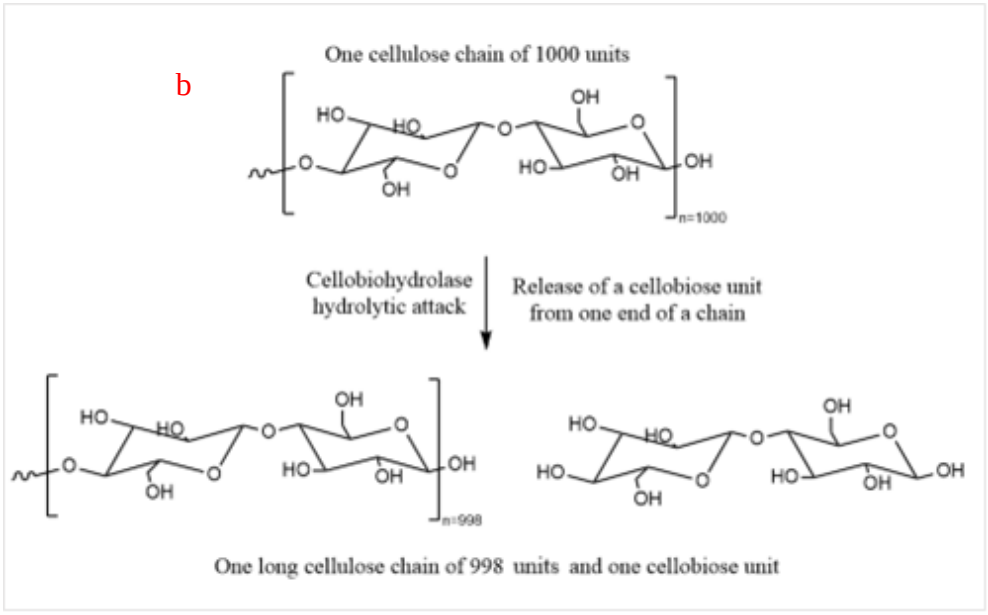


Figure 2.1.1.2. Schematic for hydrolysis of cellulose by a) endoglucanase and b) cellobiohydrolase and c) hydrolysis of cellobiose to glucose by beta-glucosidase.

Cellulase enzymes can be obtained from a variety of sources with one of the most prominent being the *Trichoderma reesei* fungus. The total culture of this species produces at least four EGs, two CBHs, and one BG. Of these, the two exoglucanases (CBH I and CBH II) are the

most abundant proteins. Of the four EGs, EG I and EG II are the most abundant, making up about 5-10% of the total culture [26].

2.1.2 Enzymes and Biodegradation

Enzymatic hydrolysis is highly important for biodegradation, but enzymes cannot accomplish biodegradation alone because it requires the action of a living organism. However, it is important to recognize the relationship between enzymes and microorganisms. Enzymatic hydrolysis is typically a first step in polymer hydrolysis, catalyzing biodegradation through secretion from a microorganism onto a substrate. The same way humans break down food through digestion with the aid of enzymes, microorganisms break down their food (in this case, cellulose) using cellulase enzymes. The enzymes facilitate hydrolysis of cellulose into glucose, which can be ultimately digested by the microorganism into byproducts like water, carbon dioxide, and methane [9]. Though the enzymes are carrying out a major portion of the macroscopic degradation, the action of the microorganism to secrete the enzymes is required for a biotic degradation scheme. Because of this relationship, enzymatic degradation experiments have been carried out in lab experiments as an alternative to microbial degradation [27].

2.2 Textile Hydrolysis

2.2.1 Substrates and Methodologies

2.2.1.1 Substrates and Success

Cellulosic textile substrates varied between published studies, as there is no standardized positive control or protocol for enzymatic hydrolysis testing of textiles. Though substrates like Whatman No. 1 filter paper or Avicel exist as common and consistent cellulosic substrates, there is no established method under which they are used and compared to other substrates for enzymatic hydrolysis. Most textile studies investigated cotton fabrics [28–30], showing either moderate

weight loss of untreated fabric pieces or high conversions to glucose after chemical or mechanical pretreatment. Cotton/polyester fiber blends were also tested to either separate fiber types for polyester recycling [22] or create lightweight PET fabrics [31]. While Strusczcyk et al. showed minimal cotton removal from 67/33 PET/cotton fabric samples, they showed up to 66% cellulose degradation from defibered samples [22]. Vasconcelos et al. used much more aggressive mechanical agitation in their PET/cotton blend separation trials, achieving 45% cotton removal (based on weight loss) after 9 hours of treatment of fabric samples, yielding intact, cleaned PET fabrics [31]. A 100% cotton fabric treated under the same conditions achieved 100% weight loss after 6 hours, showing that the presence of PET creates a physical barrier around cellulose that blocks it from being exposed to enzymatic attack. Some non-cotton cellulosic textile fibers were also investigated by Schimper et al. 2004 who showed that viscose had more efficient hydrolysis than cotton while lyocell showed less hydrolysis than cotton [26]. This was discussed as a result that would require more research because it was considered surprising for cotton to degrade more than lyocell based on their known crystal structures, where cotton should be more crystalline than lyocell [26].

2.2.1.2 Methodology Variation

While methodologies varied significantly between studies, the most consistent parameters were pH, temperature, and time. Most incubations were run at a pH within the range of 4.5-5 using a sodium acetate buffer, though some used citrate buffer [23, 32]. Most reactions were run for 24 hours, but some extended up to 50 hours [22, 33], 72 hours [23], and 96 hours [29]. Most reactions were held at a constant temperature around 45-50ºC. Temperature and pH were consistently reported as being controlled by the optimal conditions for enzyme activity. It is likely that the

treatment times were all at or below 96 hours because each study intended to perform efficient enzymatic hydrolysis, so extended time scales would not be optimal for that goal.

Liquor ratio, sometimes described as percent solids or substrate concentration [22, 23, 29, 33, 34], was more inconsistent across studies. Those claimed specifically as liquor ratios were commonly typical of textile processing, like 20:1 [29] or 30:1 [32]. Percent solids and substrate concentration values had much more divergence from typical ranges, with variation up to 100:1 [23]. Enzyme activity doses are difficult to compare because the studies all used different means of reporting. About half described their doses based on activity unit per volume, while the rest used unit per gram of fabric, with the enzyme unit being reported in either milligrams of protein or activity units. Some reports defined enzyme activity in both CMCU and FPU based on CMC viscosity-reduction assay and filter paper/reducing sugary assay, respectively, for their enzyme mixture [22, 26, 33], while others reported one or neither of those measurements (Table 2.2.1.2).

Table 2.2.1.2. Summary of cellulase enzymes used, their suppliers, reported enzyme activities, and the respective substrate tested.

			Activity		
Study	**Enzyme**	**Supplier**	**Reducing Sugar**	**CMC**	**Substrate**
Czilik et al. 2002	Celluclast 2L	Novozymes	49.08 U/mL		Plain woven bleached cotton fabric
	Novozym 188		3.37 U/mL		
	Pulpzym		32.33 U/mL		
Choe et al. 1997	Denimax Acid XCL	Novozymes		1500 U/g	Various cotton fabrics
de Assis et al. 2018	Cellulase				Wood pulp
Jones et al. 2013	Cellic® CTec2	Novozymes	139 U/mL		Hardwood pulp
Kluge et al. 2019	Several EG and BG formulations	Megazyme			Avicel
Kuo et al. 2010	Cellulase AP3 from *Aspergillus niger*	Amano Enzyme	8.5 FPU/g		Waste fabrics (100% cotton and 40/60 PET/cotton blend)
Nagl et al. 2022	Cellulase formulations				Softwood and hardwood pulp
	FiberCare R	Novozymes			
Olsen et al. 2016	Cellulase from *Aspergillus oryzae*				Avicel PH 101
Pellegrini et al. 2018	Synergistic mixture from *Th*Cel7A and *Th*Cel7B				Whatman No. 1
Schimper et al. 2004	Total culture from *T. reesei*	Genencor International		3100 U/g	Woven cotton, lyocell, modal, and viscose fabrics
Struszczyk et al. 1994	Multifect	Cultor	101 U/mL	7590 U/mL	70/30 PET/Viscose fabric
	Viscozyme	Novozymes	16 U/mL	454 U/mL	

Table 2.2.1.2 (continued).

Struszczyk et al. 1997	Primafast 100	DuPont	99 U/mL	2436 U/mL	67/33 PET/cotton fabric
	Cellulase from *T. reesei*		123 U/mL	4023 U/mL	
Teixeira et al. 2015	EG from *Pyrococcus horikoshii* and BG from *Pyrococcus furiosus*				Celish KY-100G, *Eucalyptus* holocellulose, UKWP from pine, and sugarcane basse
	Optimash BG	Enzyme Solutions (Connell Bros Company)		223.9 U/mL	
Vasconcelos et al. 2006	Cellusoft L	Novozymes		25.8 mg/mL	100% cotton taffeta and 66/34 cotton/PET blend
Vera et al. 2023	Cellic® CTec2	Novozymes	139.7 U/mL		White cotton tshirt
	Cellic® CTec3	Novozymes	196.0 U/mL		
Yu et al. 2018	Celluclast 1.5L	Novozymes	90 U/mL	175 U/mL	Waste paper fibers
Zhai et al. 2021	Cellulase	Xindeli Biological Engineering			Microcrystalline cellulose

2.2.1.3 Mechanical Agitation

Mechanical agitation during incubation was a highly variable parameter between studies. Every study used a different machine for their incubation, which in turn changes the mechanical agitation. Some agitation methods include a shaker bath [22, 33], stir bar mixing on a hot plate [23, 35], thermomixer shaking [25], rotating incubator [29], a Rotawash machine with steel discs [31], a lab-scale dyeing apparatus with piston liquor mixing [26], and a PTFE reactor with stir bar mixing [34]. Many studies did not provide a description of sample processing after incubation, but Struszczyk et al. described a washing step with boiling water and allowing samples to air dry before filtering through a Buchner funnel [22, 33]. A study looking to isolate cellulose nanocrystals (CNCs) clarified samples through centrifugal washing [32].

It was apparent that mechanical agitation during incubation is a known necessity for hydrolysis, as all methodologies included stirring or shaking during enzymatic reactions. Vasconcelos et al. intentionally tested the impact of mechanical agitation in their study using control samples that were treated without the addition of steel discs which were used as agitation aids in other samples during their process [31]. The results demonstrated the importance of mechanical agitation in a way that other studies had previously assumed, showing the effect mechanical agitation (specifically, through a pounding-like mechanism) has on disintegration of large substrate pieces. It was observed that a more intense pounding agitation (addition of more steel discs) was positively correlated with both total and insoluble weight loss [31].

2.2.2 Pretreatments

Many methodologies describe a pretreatment before enzymatic hydrolysis begins, with varying techniques being implemented. This includes mechanical or physical pretreatment like reducing sample physical size to increase surface area or pretreatment in steam or dry heat to

induce fiber swelling and increase access of the enzyme to glycosidic linkages [26]. Other pretreatments included chemical processing using different theoretical approaches to increase the conversion of cellulosic waste or biomass into glucose. Most commonly, these treatments reduced cellulose crystallinity by chemical disruption using phosphoric acid [35], sodium hydroxide [2, 36], or N-methyl-morpholine-N-oxide [NMMO] [37]. All crystal-targeting chemical pretreatments came from studies that intended to use cellulosic waste substrates as a glucose source, so the reduction of crystalline regions was able to increase conversion into glucose in each case. Alternatively, treatment of a waste substrate with polyacrylamides was shown to increase enzymatic hydrolysis through a "bridging" effect, where the polyacrylamides did not alter the chemistry of the cellulose chains but rather caused higher protein adsorption onto the cellulose substrate [34]. The ultimate disposal or reusability of the polyacrylamides was not discussed further but would be an important consideration before implementation.

Cellulosic substrates are known to enzymatically hydrolyze more efficiently with increasing surface area, which has been repeatedly confirmed in terms of both fiber size [38, 39] and particle size [23] for cotton substrates. Because of this effect, many studies include some level of pretreatment like cutting fabric into controlled swatch sizes [22, 28] or shredding into fiber [22]. Several studies did not mention any substrate preparation but given basic understanding of reaction-to-surface-area relationships, it seems probable that some mechanical deconstruction was carried out before testing. Recently, one group extensively investigated the effect of particle size reduction as a mechanical pretreatment for cotton fibers through mechanical refining, mimicking the success of other groups investigating non-cotton cellulosic substrates like hardwood cellulosics [40], or other lignocellulosic biomass sources [41, 42]. Through several mechanical size-reduction steps including cutting, grinding, disintegrating, and refining, the crystals in a cotton t-shirt were

effectively destroyed through fiber length reduction, surface fiber fibrillation, and internal delamination [29]. This extensive mechanical preparation allowed for 96% conversion of the refined cotton to glucose in 96 hours of enzymatic treatment.

However, these investigated chemical pretreatments and this extensive mechanical pretreatment increase the overall processing time and energy input required to convert cellulose to glucose. Of course, to achieve high levels of conversion to glucose using cellulase enzymes, the cellulose crystals need to be disrupted. However, potential recycled use for crystalline cellulose in composites or as feedstock for regenerated cellulose fiber spinning [15] could provide incentive against crystal reduction from cellulosic substrates. In this case, recalcitrant and highly ordered cellulose fiber fragments could be isolated for reuse while removing the need for chemical or intensive mechanical pretreatment.

2.2.3 Recalcitrant Fiber Product

Commonly, the reviewed enzymatic degradation results from fabric samples are quantified in percent total weight loss of the substrate. The idea of a small solid portion or partially degraded, but still insoluble, substrate was not reported or described in depth, though mention of a recalcitrant fiber degradation product was present in multiple reports. One group described their degradation product as a slurry but was only interested in the fermentable sugars present in the liquid component, so disregarded the non-soluble portions of their product in results reporting [34]. Further, a discussion of enzymatic fiber separation mentioned cellulose fragmentation as a mechanism that allows for full isolation of PET during post-incubation washing [33], though there was no quantification or characterization of the fiber fragments beyond that for textile substrates. A further study quantified insoluble weight loss, described as the weight obtained after filtration of treatment solution [31] in their effort to remove cotton from a PET/cotton blend. It is said that

cellulose degradation is dependent on the product of insoluble material, which could be referring to highly crystalline solids that do not fully convert to soluble sugars during the enzymatic reaction.

Kluge et al. used EG and BG cellulases in their enzyme formulation to avoid formation of cellobiose and reported 75-86% yield of cello-oligomers with a weight average degree of polymerization (DP_w) of 65 [25], which is an insoluble cello-oligomer product. These oligomers likely represent fine fiber fractions obtained at a high yield from Avicel, which could be attributed to the EG used for degradation. However, the solid product is only described in terms of its degree of polymerization, which was measured using solubilized solutions in dimethylformamide (DMF) through gel permeation chromatography.

2.3 Characterization and Potential Use of Degradation Products

2.3.1 Degradation Product Characterization Techniques

Because many studies aimed to generate glucose from cellulosic biomass or textiles, clear preferred methods of glucose content quantification were apparent. Some studies quantified glucose release from a sample by determining the glucose concentration of a liquid aliquot through reducing sugar assay [22], but most used HPLC detection [23, 29]. Though enzymatically cleaned PET samples were not paired with analytical techniques, PET has useful characterization techniques in Fourier Transform Infrared analysis [FTIR] [43], thermogravimetric analysis [TGA] [44], and differential scanning calorimetry [DSC] [45] for quality analysis.

Because characterization of recalcitrant fibers as a degradation product was not found in reviewed studies, common textile and fiber analytical techniques for analysis of crystallinity and degradation characterization were investigated. Fiber Quality Analysis [FQA] has been used to determine the average fiber length of partially degraded fibers from enzymatically treated paper waste fibers [46]. More qualitative analyses have been carried out on partially degraded fabrics or

substrates using Scanning Electron Microscopy [SEM] to look for surface fiber damage and observe cellulose fragmentation [29, 31]. Crystallinity of cellulose has been determined in previous hydrolysis studies to show crystallinity had decreased after chemical [47] or mechanical [29] pretreatment, which was quantified by the Segal method using X-ray diffraction [XRD] [48]. Some groups also examined crystallinity using FTIR [29, 43], but XRD was much more common. In characterizing a fine fiber degradation byproduct for potential renewed use, crystallinity would be an important property to understand.

2.3.2 Recycling Pathways for Cotton and Polyester Cellulase Hydrolysis Products

Wang and Salmon compiled a comprehensive review of new recycling technologies that are emerging for cotton and PET textiles, as well as breaking this heading down into technologies that exist for different degradation products after mechanical or chemical recycling of the two fibers [15]. Some highlights include the use of mechanically recycled cotton fibers as feedstock for regenerated cellulosic fibers [49] or cellulose nanocrystals (CNCs) [50] or as reinforcement agents in polypropylene composites [51]. Interestingly, the composite reinforcement end use is described as a high-value application [15] that uses dyed cellulose fibers as their reinforcing agent [51], which significantly opens the doors for textile recycling. If recycled fibers do not require decolorization for their end use, then a dye removal step could be avoided for some circular pathways, and emerging recycling technologies could develop strategies to overcome dye or other chemical obstacles without removal, which could possibly reduce chemical input. Soluble cotton hydrolysis products, like glucose, are useful feedstocks for biofuel fermentation [50]. Other cotton fiber and sugar byproduct valorization pathways include as feedstock for downcycled applications like composting [27], which could promote enhanced soil health, and anaerobic digestion for biogas generation and energy production [52]. Polyester has an established mechanical recycling

pathway based on its inherent thermoplastic quality, and current research commonly focuses on chemical recycling pathways: converting the polymer back into monomeric components for the sake of repolymerization into high quality PET [15]. Some monomer conversion techniques for PET include alcohololysis [53] and biological pathways using newly developed enzymes and microbes [54] to yield monomeric components like terephthalic acid, ethylene glycol, and bis-hydroxyethyl terephthalate [BHET], an oligomeric PET unit [15].

2.4 Chemical Obstacles to Degradation

2.4.1 Effect of Dyes

Preliminary research on enzymatic hydrolysis is enough to back up that cotton can be effectively hydrolyzed in controlled enzymatic conditions. However, there is minimal research on degradation of dyed cotton under those same enzyme treatment conditions. For enzymolysis to be considered a viable circular alternative for cotton textile disposal, realistic fabric types must be tested and understood in terms of their limitations, degradation products, and any corresponding toxicity in its future in the supply chain.

Most older work on cellulase hydrolysis of cotton fabrics focused on stonewashing using enzymes rather than full degradation of fabrics [55–57]. Though only investigating surface effects, influence of dye on the extent of stonewashing was repeatedly tested. Compared to the undyed control samples, dyed fabrics commonly showed decreased surface abrasion. Dye class was shown to play a major role, where fabrics dyed with reactive dyes and direct dyes showed a major effect compared to fabrics colored with vat dyes [55, 58], which were considered to not show significant effects on the abrasion performance. While reactive dyes are covalently fixed to the polymers, vat dyes are typically considered to only be present in between the polymers within the fiber, so would have a smaller molecular hinderance on surface erosion than a reactive dye. Reactive dyes were

examined in detail based on their functionality, and it was repeatedly reported that bifunctional reactive dyes caused a more significant impediment than monofunctional reactive dyes [55, 56, 59], typically attributed to fiber crosslinking, which was previously confirmed through nuclear magnetic resonance spectroscopy [60].

Supporting these effects seen in biopolishing trials, a group investigated enzymatic hydrolysis of fibers dyed with different reactive dyes to examine the reaction kinetics over time during complete cellulose degradation. Two MCT-VS bifunctional reactive dyes were investigated. In comparison to an undyed fabric that achieved 85% weight loss after 24 hours, the two dyed fabrics of Dye A and Dye B showed 43% and 38% weight loss, respectively. The chemical structures for each dye were provided, but they were not named through or dye number or Colour Index identification. This study also showed that over time, rate of enzymatic hydrolysis decreased for the dyed fabrics, and suggested it was because of fiber crosslinking by the dye molecules [30].

2.4.1.1 Dye Removal

Because dyes are seen as a major obstacle to cellulose hydrolysis, removal of dye from a textile before enzymatic hydrolysis studies is common [15]. Especially for groups looking to use glucose from cotton as a source for biofuels, dyes are removed to increase the purity of the glucose sample as well as reduce the reaction time needed to create glucose from cellulose [14, 35]. To illustrate this, consider direct dyes. This dye type is known to adhere to cellulose through hydrogen bonding, but it is also known that the dye molecules, being planar, typically settle in between the beta-sheets present in the secondary structure of cellulose [61]. Further, because direct dyes are so large, they interact with many saccharide rings and glycosidic linkages along the polymer backbone, blocking access of large proteins like enzymes to break them down. Because of the decreased access to the polymer backbone, enzymes cannot degrade dyed cotton fabrics as easily

as bleached cotton. So, the removal of dyes from a cotton substrate can increase the accessibility of microorganisms to the polymer backbone, allowing for more rapid hydrolysis [50]. If dye removal steps can be avoided because of hydrolysis processing optimization, the viability of enzymatic hydrolysis as a textile waste strategy could be increased through reductions in energy, time, and chemical inputs.

2.4.2 Durable Press Finish

Another common chemical additive is a durable press finish, which is used to impart wrinkle resistance on typically woven cotton textiles like dress shirts, but the finish can also be applied to any cellulosic substrate. Because cellulose polymers are loosely held together by hydrogen bonds, water disruption through laundering can cause polymer and fiber displacement. Once the fabric is dried in the disrupted state, wrinkles form in areas of displacement. To combat this, a crosslinking chemical (commonly, dimethyloldihydroxyethylene urea [DMDHEU]) is used to form covalent links between cellulose polymers [62]. This covalent bond prevents any displacement due to hydrogen bond breakage during laundering. As with bifunctional reactive dyes, it is expected that this crosslinked fabric would inhibit enzymatic degradation of the polymers [57]. This effect was observed in a recent biodegradation study that investigated soil burial behavior of cotton fabrics with multiple common finishes, including DMDHEU crosslinking. It was observed that the control unfinished cotton fabric showed about 60% weight loss while crosslinked fabric showed about 20% buried in soil for 160 days, so there was a major decrease in degradation efficiency [63].

Though enzymatic degradation studies investigating the crosslinked finish were not found, a group who looked at biopolishing of crosslinked fabrics reported that the level of hydrolysis achieved was impacted by the level of crosslinking [57]. Perhaps this chemical finish imparts a

high level of interference on enzymatic hydrolysis, which could require the use of a chemical pretreatment to mitigate or remove the crosslinking obstacle. One strategy to mitigate this obstacle is increasing enzyme access to glycosidic linkages through alkaline treatment, which has been shown previously to reduce crystallization and cause fiber swelling [2, 36]. Another strategy is the removal DP crosslinks from a fabric through acid hydrolysis which has been previously shown to sever DMDHEU crosslinks [62]. Perhaps the development of a chemoenzymatic pathway for degradation of highly degradation-resistant textile substrates could provide a positive pathway for the future of the implementation of this technology.

3. METHODS

3.1 Raw Materials

Fabrics were purchased from Testfabrics, Inc. (West Pittston, PA, USA), including: bleached 100% cotton t-shirt fabric (Style 437W, jersey knit, basis weight 149 g/m2); 50/50 cotton/polyester fabric (Style 7422, jersey knit, basis weight 172 g/m2); and, spun viscose challis (Style 266, woven, basis weight 138 g/m2). A commercial 55/45 modacrylic/cotton khaki dyed interlock knit and 55/45 modacrylic/cotton navy dyed, durable press finished twill weave were provided by Kaneka Corporation (Pasadena, TX, USA). Reactive Blue 19 (Permabril Brilliant Blue R) and Reactive Red 198 (Permabril Red RB) dyes were obtained from Standard Colors, Inc. (High Point, NC 27263 USA). Dyeing auxiliaries Fumexol and Apolloscour were obtained from Huntsman Corporation (The Woodlands, Texas, USA) and Apollo Chemical Company (Burlington, NC 27215), respectively. Other dyeing auxiliaries, sodium sulfate, and soda ash came from Brenntag North America, Inc. (Reading, PA 19605 USA). The crosslinking agent dimethyloldihydroxyethylene urea (DMDHEU) and crosslinking auxiliary Catalyst KR were obtained from Omnova Solutions (Beachwood, Ohio 44122 USA). The wetting agent Leonil EH was obtained from Archroma (Pratteln, Switzerland). Cellic® CTec2, a formulated liquid product containing multicomponent cellulose-degrading enzymes as well as several experimental monocomponent enzymes and an additional multicomponent enzyme, were provided by Novozymes North America, Inc. (Franklinton, NC 27525 USA). Whatman No. 1 filter paper was purchased from Sigma-Aldrich (St. Louis, MO 63178 USA). Glass fiber filter circles (Fisherbrand™ Glass Filter Circles G6; 55mm diameter; 1.6μm particle retention) and the reagent grade chemicals sodium acetate (CH_3COONa), glacial acetic acid (CH_3COOH), sulfuric acid (H_2SO_4), and 50% sodium hydroxide (NaOH) were purchased from Fisher Scientific (Waltham,

MA 02451 USA). Stainless steel sink drain filters with 2mm mesh screen openings were purchased from a local retail store. Kimble 14395-250 GL-45 glass bottles with blue polypropylene screw caps, 50mm straight and 40mm PTFE egg-shaped stir bars were purchased from Amazon (www.amazon.com). Except as otherwise described herein, all materials were used as received. These raw materials were previously listed in a prior publication [64].

3.2 Fabric Preparation Methods

Fabric dyeing, finishing, and laundering were carried out using equipment in the Dyeing and Finishing Laboratory at the Wilson College of Textiles in Raleigh, North Carolina. Many methods in sections 3.2 through 3.7.1 were previously published in a prior article [64].

3.2.1 Fabric Dyeing

Exhaust dyeing of Reactive Blue 19 and Reactive Red 198 on cotton fabrics was performed in a Mathis jet-dyeing machine (Werner Mathis AG, 8156 Oberhasli, Switzerland) with a 10:1 liquor ratio. Sodium sulfate (60 g/L) and an anti-foaming agent (Fumexol) were added initially. Then dye (3% owf) and soda ash (15 g/L) were dissolved in water and added incrementally, with the second half of the soda ash added after the dye bath reached the dyeing temperature. The bath and fabric were heated up to 60°C at 2°C/min and cycled for 60 minutes before draining and rinsing to remove excess surface dye molecules. A post-scour was carried out with surfactant (Apolloscour SDRS, 1.5% owf) and acetic acid (0.75% owf) to remove unfixed dye.

3.2.2 DMDHEU Crosslinking

DMDHEU durable press was applied at two different concentrations to simulate the “standard” commercial dose and a “half” dose. Auxiliary components were added either at the standard dose listed here for DP fabric or at half of the listed dose for 0.5DP fabric. Before processing, woven cotton leader fabrics were sewn onto each end of the primary knit fabric. Fabric

was soaked in a 5L bath containing 6% Permafresh 600 (glycolated DMDHEU crosslinker), 2% catalyst KR, and 0.2% wetting agent (Leonil EH) for about three minutes before being fed through a Greenland padder that was set to achieve 100% wet pickup. Immediately afterwards, fabric was sent through a tenter frame to dry at 135°C and was then cured at 165°C for 90 seconds to induce crosslinking reactions.

3.2.3 Fabric Laundering

The two dyed fabrics, the DP finished fabric, the 50/50 polyester/cotton blended fabric, and a portion of the original white 100% cotton knit were laundered to simulate wear that is expected of post-consumer textile waste. To avoid curling, fabric was cut into approximately $1m^2$ square pieces, folded in half, and the edges were sewn prior to washing/drying. Each different fabric type was separately washed/dried 10 times according to the AATCC LP1-2021 test method for home laundering [65] using AATCC standard powder detergent in a GE top-loading washer and a Kenmore dryer.

3.2.4 Fabric Characterization Methods

3.2.4.1 Burst Testing

Burst Testing was carried out in the Wilson College of Textiles Physical Testing Laboratory according to ASTM D3786 using a James H. Heal & Co. Lt.d TruBurst^2 unit (Halifax, England) on all washed and dried fabrics samples, which were allowed to condition to 65% relative humidity and 72 degrees Fahrenheit for 24 hours before testing.

3.2.4.2 Fabric Count

Courses per inch and wales per inch of each fabric type were measured according to ASTM D8007 on all knit fabric types.

3.2.5 Fabric Nomenclature

Tested fabrics were assigned two-letter acronyms that were used in results reporting. Fabric labels and relevant specifications are summarized in Table 3.5.2.

Table 3.2.5 Fabric acronyms and descriptions

Acronym	Translation	Description
DC	Delta Cotton	100% cotton waste tshirt, assumed to be bleached white cotton
UC	Untreated Cotton	100% bleached white cotton jersey knit, as delivered from Testfabrics Inc. Item Number 437W
BC	Blue Cotton	100% cotton from Testfabrics Inc. (437W) dyed to a 3% shade with Reactive Blue 19 (see 3.2.1)
RC	Red Cotton	100% cotton from Tesfabrics Inc. (437W) dyed to a 3% shade with Reactive Red 198 (see 3.2.1)
DP	Durable Press	100% cotton from Testfabrics Inc. (437W) finished with industry standard dose of DMDHEU and Catalyst KR (see 3.2.2)
0.5DP	Half Durable Press	100% cotton from Testfabrics Inc. (437W) finished with a “half” industry standard dose of DMDHEU and Catalyst KR (see 3.2.2)
PC	Polyester/Cotton	50/50 PET/cotton blended jersey knit fabric as received from Tesfabrics Inc. Item Number 7522

Table 3.5.2 (continued).

KK	Khaki Knit	Khaki dyed (unknown dye) interlock knit 55/45 modacrylic/cotton blended commercial fabric from Kaneka
NP	Navy Pants	DMDHEU-crosslinked, navy dyed (unknown dye), 55/45 modacrylic/cotton twill weave flame retardant work pants from Kaneka
VI	Viscose	Viscose challis fabric as received from Testfabrics Inc. Item Number 266

3.3 Reducing Sugar Assay

A variation of the Filter Paper Unit (FPU) assay for measuring cellulase activity, using a modified version of the standard reducing sugar assay [66] with dilution and color-developing solutions scaled down to volumes suitable for use with 96-well microtiter plates, was used to measure CTec2 enzyme activity. The standard citrate buffer was replaced with 10mM sodium acetate buffer (pH 5.0). Absorbance measurements on color-developed solutions were made at 540nm using a TECAN Spark spectrophotometer (TECAN Group Ltd., CH-8708 Männedorf, Switzerland) and compared to a calibration curve made with solutions of known glucose concentration, as described in the method. The nominal activity for Cellic® CTec2 was measured to be 172 FPU/mL (see Table 4.2.4.1 below).

3.4 Enzymatic Degradation Methods

3.4.1 Sample Preparation

Fabrics were cut into approximately 1cm^2 square swatches to simulate chopped waste fabric material. Approximately 1g of fabric swatches (exact weights recorded to +/- 1 mg) was weighed and transferred into a 250mL GL-45 glass bottle. The corrected fabric dry weight was

calculated and used to determine the combined amounts of buffer and enzyme solutions needed to achieve a final 20:1 liquid-to-solids ratio in the bottle (equivalent to a 5% solids concentration).

3.4.2 Enzymatic Degradation

Fabric samples were treated with nominal Cellic® CTec2 enzyme doses of 0, 0.2x, 1x, 2x, and 4x, and each treatment was carried out in triplicate, unless otherwise indicated. The nominal doses translate to percent of enzyme on weight of goods, where the "x" represents 5%, so the 1x dose is 5% enzyme on weight of fabric. One 40mm x 13mm egg-shaped PTFE stir bar (12g) and one 50mm x 4mm straight PTFE stir bar (11g) were added to each bottle containing fabric, buffer and enzyme (or without enzyme for control treatments). Bottles sealed with screw caps were placed in a magnetic-stirred temperature-controlled block (2mag Stirring Drybath 15-250, 2mag-USA, Daytona Beach, FL 32121 USA) where they were incubated at 50°C and 500rpm for 19 hours, unless otherwise indicated.

3.4.3 Sample Processing

Bottle contents were further processed through a sequence of filtration and washing steps into three separate fractions: (1) large solids (> 2mm), (2) small solids (< 2mm), and (3) clarified liquid. First, any large solids remaining after hydrolysis were separated through simple solid/liquid separation by pouring the contents of the bottle over a 2mm mesh screen mounted on a clean dry 250mL beaker. Next, for samples requiring glucose concentration analysis, small solids were separated by vacuum filtration without diluting the incubation liquid. The contents of the 250mL beaker were poured over a pre-weighed G6 glass fiber filter held in a Buchner funnel on a clean dry 50mL glass side arm flask, with vacuum applied by a vacuum pump. The clarified liquid filtrate was transferred to a clean labeled screw cap centrifuge vial and frozen at -30°C until needed for analysis. The G6 filter covered with a collection of damp small solids was carefully removed from

the funnel and placed on a labeled petri dish to air dry. Any large solids previously collected on the 2mm screen were placed back into the original GL-45 sample bottle with about 40mL of hot water. The mixture was shaken to wash small solids from residual large fabric pieces and to remove residual fibers from the walls of the sample bottle.

This “first wash” was poured over the 2mm screen and into the beaker. The “washing” step was repeated a total of three times for each sample. After the third wash, any remaining large solids were removed from the 2mm screen with tweezers and spread on a labeled glass petri dish to air dry. The liquid and small solids that passed through the screen during the washing steps (the “diluted slurry”) were collected in the beaker and then were separated by vacuum filtration by pouring the slurry over another pre-weighed G6 glass filter as previously described. The “wash” liquid filtrate from the diluted slurry was disposed.

The G6 filter with a cake of damp small solids was carefully removed from the funnel and placed on a labeled petri dish to air dry. Any large solids fabric pieces and the G6 filters with small solids cakes were air dried overnight and then dried separately in the moisture analyzer to obtain large and small solid product dry weights, after subtracting the weight of the respective G6 filters. G6 filters were selected for their filtration efficiency and low ambient moisture content of around 1%, which was considered negligible in gravimetric calculations. Percent weight loss (Section 2.2.2) was calculated to measure the extent to which fabrics were converted to water soluble products by enzymatic hydrolysis.

3.4.4 Double Treatment

Some fabrics were treated with two sequential enzyme treatments to achieve full degradation. In these cases, after the first 19-hour treatment, the degradation products were separated into large and small solid fractions as described above, including washing steps. The still

wet large solids were immediately returned to the sample bottle with fresh enzyme and buffer solutions for a second 19-hour treatment. The enzyme amount used in the second treatment was the same as used in the first treatment (based on initial undegraded sample corrected dry weight). After the second treatment, fabric samples were processed and collected as normal.

3.5 Moisture Analysis

3.5.1 Sample Preparation

Fabric moisture content was measured by drying samples of cut fabrics in a moisture analyzer (Mettler Toledo HC103, Mettler-Toledo, LLC, Columbus, OH 43240 USA) at 105°C. Analysis was programmed to stop after the sample mass decreased less than 1mg after 50 seconds of drying. The percent moisture content, averaged from two samples, was used to calculate corrected dry weights for individual fabric samples that were weighed at ambient conditions.

3.5.2 Gravimetric Analysis

Residual large solids (m_L) and small solids (m_S) dry weights after treatment and initial fabric dry weights (m_i) were used to calculate the percent weight loss of fabric (Equation 3.5.2.1) after treatment. Percent weight loss values were converted to glucose concentrations using Equation 3.5.2.2, where 1.11 is the dehydration factor for glucan to glucose.

$$\%\ Weight\ Loss\ = \frac{m_i - \left(m_L + m_S\right)}{m_i} \cdot 100$$

Equation 3.5.2.1

$$Glu\left(\frac{g}{L}\right) = \frac{\%\ Weight\ Loss}{100} \cdot 1.11 \cdot \frac{1000\ mL}{1\ L} \cdot \frac{1}{Liquor\ Ratio}$$

Equation 3.5.2.2

3.6 Chemical Pretreatments for DP Finished Fabric

3.6.1 Cold alkali Pretreatment

The Gholamzad et al. [36] alkali pretreatment was applied to fabric swatches as follows. Precut swatches were soaked at a 20:1 liquor ratio in a 12 wt% NaOH solution for one hour at -30°C with swirling every 10 minutes. After treatment, swatches were washed in deionized (dI) water to neutralize the pH and then were washed in 10mM sodium acetate buffer solution (pH 5.0) to adjust the fabric to the enzymatic treatment pH. Swatches were air dried before enzymatic treatment, and corrected dry weights were used to calculate enzyme dosing and buffer amount. This means any weight loss that may have occurred during chemical pretreatment was not accounted for in the final gravimetric analysis.

3.6.2 Acid/Alkali Pretreatment

An adaptation of the Haule et al. [67] sequential acid/alkali method was carried out by combining the referenced acid treatment together with the cold alkali treatment above. Fabric swatches were first soaked for 2 hours at room temperature in a sulfuric acid solution (10 mL/L) at a 10:1 liquor ratio before heating to 60°C and soaking for another 30 minutes. After the acid treatment, fabric was removed, squeezed of excess liquid, rinsed briefly with dI water, squeezed of excess liquid, placed in a 12 wt% NaOH solution at a 20:1 liquor ratio, and treated and washed according to the cold alkali method.

3.6.3 Calculation of Weight Loss after Acid Pretreatment

The initial mass of treated fabric was measured and corrected for dry weight according to the method described in Section 3.5.1. After acid treatment, fabric was allowed to dry overnight and final weight was obtained and corrected by the same method as the initial weight, using

moisture content of the treated fabric. Then, weight loss was calculated according to Equation 3.6.3 where m_i and m_f are the initial and final dry weights, respectively.

$$Weight\ Loss\ (\%) = \frac{m_i - m_f}{m_i} \cdot 100$$

Equation 3.6.3

3.7 Degradation Product Characterization Methods

3.7.1 LC-CAD Glucose Analysis

For liquid fraction analysis described in Section 4.4, glucose released from enzymatic hydrolysis was measured by an Agilent 1200 series modular HPLC with a refractive index detector (Agilent Technologies, Santa Clara, CA, USA). Separation was performed at 65ºC with a 7.8mm × 300mm Aminex HPX-87H column, (Bio-Rad Laboratories, Hercules, CA, USA). Separations were run through isocratic elution with a mobile phase of 5mM H_2SO4 at a flow rate of 0.6mL/min. Glucose was quantified against an external calibration standard between 0.01–15.00mg/mL.

Liquid chromatography with charged aerosol detection [LC-CAD] was used for glucose analysis of experiments described in Section 4.4.1. Samples were prepared by adding 900µL acetonitrile to 100µL of sample to precipitate residual enzymes by centrifugation (5 min). Aliquots of 25µL of the supernatants were then analyzed by LC-CAD (Ultimate 3000 UPLC/Charged Aerosol Detector, Thermo Fisher Scientific). The instrument was also equipped with a UV/vis Diode Array Detector (DAD) and a mass spectrometry detector (ISQ EC). A volume of 25µL was injected and separated on a Waters Acquity BEH Amide column (1.7µm × 2.1cm × 15cm) with matching guard column. Separations were done with an isocratic solution using 25% water/75% acetonitrile/ 0.1% ammonium hydroxide over 7 min at a flowrate of 0.5 mL/min and column temperature of 35ºC. The first 1.5 min were diverted to waste to desalt the sample. Glucose

standards dissolved in 90% acetonitrile were injected at the beginning and end of a sequence in the range of 0.1–10mM. The glucose peak was observed at 2.34 min and quantified by peak area in the Charged Aerosol Detector. A linear calibration curve was used to calculate glucose concentrations in the samples.

3.7.2 Fiber Quality Analysis [FQA]

Fiber length of small solids collected from time-response trial samples was measured by an OpTest Equipment Inc FQA-360 (Hawkesbury, ON, Canada). A small amount of slurry content from each time-response sample was added to the sample beaker, and then the testing beaker was automatically filled to about 500mL for testing. Testing concluded after 10,000 fibers were measured, and the flow cell was automatically purged between each measurement. Weighted length was used instead of arithmetic average for fiber length comparison because it places more emphasis on the longer fibers and reduces the impact of fines.

3.7.3 X-Ray Diffraction [XRD]

Small solids samples from the time-response trial were analyzed using X-ray diffraction on a Rigaku SmartLab X-ray Diffractometer (Tokyo, Japan). Method settings were: v = 40 kV, I = 44 mA, CuK α1 radiation source (λ = 1.5406 Å), continuous scan mode, 2θ range = 10-40º with a step of 0.07º at room temperature. Crystallinity index of the samples was calculated using the fundamental method established by Segal et al. [68] using Equation 3.7.3, where I_c corresponds with the maximum intensity in the crystalline response region between 22-23º and I_a is the minimum intensity in the amorphous response region between 18-19º. Results were further analyzed using Origin 2023b.

$$CI = \frac{I_c - I_a}{I_c} \cdot 100$$

Equation 3.7.3

3.7.4 Fourier-Transform Infrared Analysis [FTIR]

Fourier-transform infrared spectroscopy [FTIR] was performed on reference and recovered synthetic solids samples using a Nicolet Nexus 470 spectrometer equipped with a Nicolet OMNI germanium crystal Attenuated Total Reflectance [ATR] sampling head (Thermo Fisher Scientific, Waltham, MA 02451 USA). A total of 32 scans at a resolution of $4cm^{-1}$ were collected for each sample in the range of 700–$4000cm^{-1}$. Each FTIR spectrum was baseline corrected and peaks were assigned using the operation software.

3.7.5 Thermogravimetric Analysis [TGA]

The thermal degradation of reference and recovered synthetic solid samples were determined by thermogravimetric analysis using a TGA 550 (TA instruments, New Castle, DE, USA). The decomposition was carried out under nitrogen atmosphere with a gas flow of 25mL/min. All samples were between 5-10 mg and were heated from room temperature to 600°C at a heating rate of 20°C per minute.

3.7.6 Differential Scanning Calorimetry [DSC]

Differential scanning calorimetry [DSC] was performed on reference and recovered synthetic solids samples with a PerkinElmer Diamond DSC-7 (PerkinElmer Inc., Waltham, MA 02451 USA) instrument purged continuously with nitrogen gas. Between 5-10mg of each sample was sealed in a DSC aluminum pan and was heated from 25ºC to 280ºC at 20ºC/min followed by a one-minute isothermal holding period at 280ºC to erase any thermal history. Samples were then

cooled from 280ºC to 25ºC at −20ºC/min and heated again from 25ºC to 280ºC at 20ºC/min. Results were further analyzed using Origin 2023b.

3.8 Statistical Analysis

3.8.1 Significant Digits

Degradation results were reported to 4 significant digits because the masses were measured on balances with a sensitivity of at least +/- 1mg. In discussion, results are rounded to 2 significant digits because they fall within the first standard deviation of the mean. Standard deviation from the mean was reported for UC, DC, and BC gravimetric degradation results. It was not reported for other fabric types because they did not provide additional discussion points, though did support the trends discussed in Chapter 4.

Significant digits reported for other data (burst testing, FQA, etc.) reflect the significant digits reported by the relevant machine and required by subsequent calculations.

3.8.2 Data Analysis

Statistical analysis was carried out with SAS JMP Pro 17, which was used to analyze general trends in degradation and characterization data resulting in correlation plots between relevant variables, which are reported in Section 4.5.1, and box plots that are reported in Appendix A-6.

4. RESULTS AND DISCUSSION

4.1 Burst Testing

Burst testing was carried out as a tensile property test to characterize the strength of the tested knit fabrics. Attention was paid to fabrics with different chemical additives, as their base fabric was the same, so changes in strength could be indicative of altered cellulosic chemistry and could later correlate with observed enzymatic degradation changes. It was shown that the average distention and average pressure at burst of the untreated cotton (UC) and blue dyed cotton (BC) fabric are very similar (14.5 and 14.3mm or 87.59 and 87.4psi, respectively, from Table 4.1). Because these are the same 100% cotton base fabric, this is expected. Other modifications of the same fabric showed different results, specifically the half-dose durable press finished cotton (0.5DP), standard durable press finished cotton (DP), and red dyed cotton (RC) fabrics. It is generally understood that the crosslinking in a durable press fabric can reduce burst strength due to an increase in brittleness. For this reason, the decreases in average pressure and distention in the two DP varieties compared to UC were reasonable. This was further emphasized by the trend between the two DP varieties where the 0.5DP required more pressure and distention (10.42mm and 50.34psi) before burst than the DP (8.3mm and 35.5psi), the latter of which was anticipated to have an increased brittleness because of the higher DMDHEU dosage. The RC fabric also showed slightly reduced distention and pressure (13.9mm and 78.1psi) to both UC and BC. This decrease could be explained by the dye structure, where the blue dye is a monofunctional dye and the red is bifunctional. The bifunctionality of the red dye likely led to crosslinking between cellulose chains [30], creating an effect similar to the fully crosslinked DP varieties, just less emphasized.

Table 4.1. Burst testing results for each fabric type, run according to ASTM D3786.

	Fabric	Average PSI	Average Distention (mm)	Inflation Rate (psi/second)
Washed	0.5DP	50.34	10.42	3.48
	DP	35.5	8.3	2.83
	RC	78.1	13.9	5.04
	KK	88.25	14.3	5.34
	PC	101	16.5	6.43
	BC	87.4	14.3	4.97
	UC	87.59	14.5	5.34

Though the blended fabrics, 50/50 polyester cotton (PC) and 55/45 modacrylic/cotton (KK), are not comparable to the 100% cotton fabrics or each other, their results are still relevant. PC required the most pressure and fabric distention before burst (101psi and 16.5mm), indicating the fabric strength to be higher than the cotton fabrics. KK interestingly showed very similar results to UC and BC fabrics (14.3mm and 88.25psi). KK fabric was the only tested fabric that was not a jersey knit, as it was a double knit. For this reason, there is not much to conclude about the results of the KK fabric in relation to the other fabrics.

4.2 Bleached 100% Cotton Fabric Hydrolysis

4.2.1 UC Hydrolysis

After 19 hours of treatment in a 2mag mixer, enzymatic hydrolysis of bleached cotton fabric was observed. It was shown that as enzyme concentration increased, so did the observed hydrolysis into soluble sugars (weight loss) and partially degraded small solids (Figure 4.2.1). This result was expected and is attributed to a larger amount of enzyme-fiber interaction at higher enzyme doses. Without enzyme, negligible degradation was observed under the same incubation and agitation conditions. The observed 1.0% small solids (Table 4.2.1) are attributed to general

microfiber shedding observed during textile processing including laundering from the interaction with the bath and abrasion from the interaction with the stir bars.

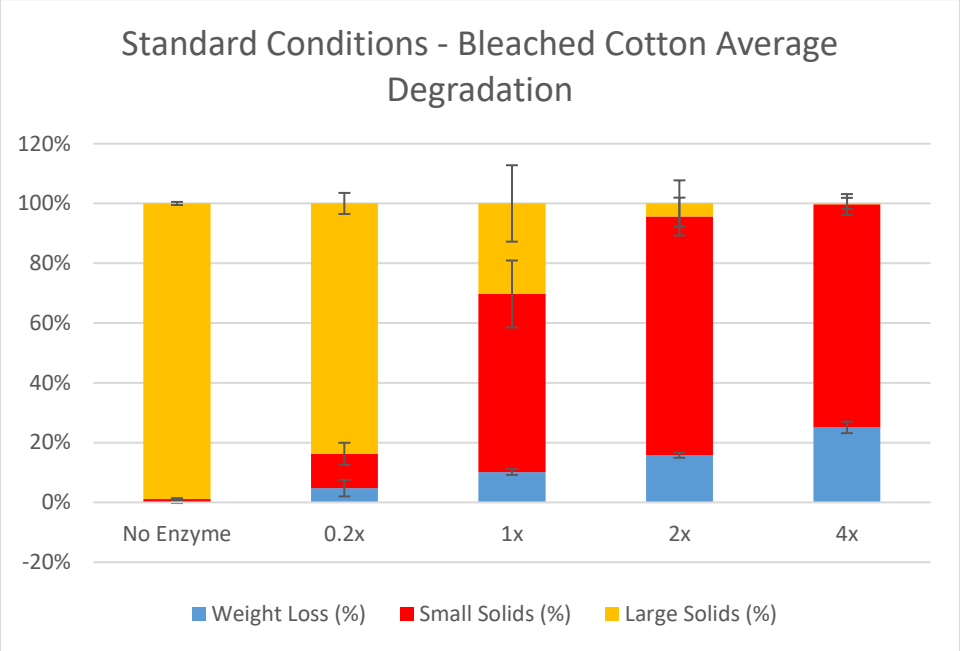


Figure 4.2.1. Average bleached cotton degradation at different enzyme doses. Error bars show the standard deviation.

Table 4.2.1. Average bleached cotton degradation at standard conditions for varied enzyme doses.

Enzyme Treatment	Weight Loss (%)	Small Solids (%)	Slurry (%)	Large Solids (%)
No Enzyme	0.10	1.03	0.84	99.16
0.2x	4.86	11.69	14.47	85.53
1x	10.21	59.80	69.59	30.41
2x	15.74	79.58	95.63	4.37
4x	24.96	74.14	99.67	0.33

Notably, the standard deviations from the average for large and small solids are larger than the standard deviation for average weight loss in UC hydrolysis data (error bars, Figure 4.2.1). So, the hydrolyzed samples typically show more consistent weight loss and less consistent fabric disintegration from large pieces into small solids for samples that have large solids remaining. This

is mainly attributed to the mechanical agitation applied during treatment, which was intentionally aggressive and characteristically inconsistent. Fabric samples were all agitated by the addition of two magnetic stir bars, which creates a vibrational "beating" agitation which expedites fabric disintegration due to enzyme interaction, which was shown to be effective previously [31]. This agitation style, unlike single stir bar mixing, is inherently random and causes inconsistent solids size reduction. Sometimes fabric pieces became stuck under stir bars and so were not sufficiently beat up, and occasionally fabric pieces were flung out of the liquid and onto the side of the sample bottle, resulting in no degradation of that piece. These infrequent occurrences partnered with the random nature of the agitation along with general error expected from enzyme hydrolysis explain the notable variation in large and small solids proportions between samples of the same treatment. For a fully degraded sample, like the 4x dose, this same phenomenon is not observed because the enzyme concentration was sufficient to disintegrate the fabric regardless of the inconsistent mixing condition.

4.2.2 Effect of Buffer Concentration on Hydrolysis

Hydrolysis experiments initially used 100mM sodium acetate buffer, but the concentration was later changed to 10mM buffer to reduce chemical oxygen demand [COD] of samples sent to anaerobic digestion facilities, which was the latter half of the project that funded this work that will not be discussed further herein. Because there is no anticipated ion release during hydrolysis that would influence pH during incubation, degradation results from experiments with different buffer concentrations were expected to be comparable. A comparison of average degradation gravimetric data is shown in Figure 4.2.2 and Table 4.2.2. Though the hydrolysis differences were minimal between the two buffer concentrations, it was seen that at the 0.2x and 1x doses, the 100mM buffer samples showed higher yield in both weight loss and small solids. For example, at

the 1x dose, 100mM samples had 10% weight loss and 64% small solids after hydrolysis while 10mM samples showed 10% weight loss and 52% small solids on average. At the 2x and 4x dose, this same effect was not observed, as the gravimetric data is comparable on average between the two buffer concentrations.

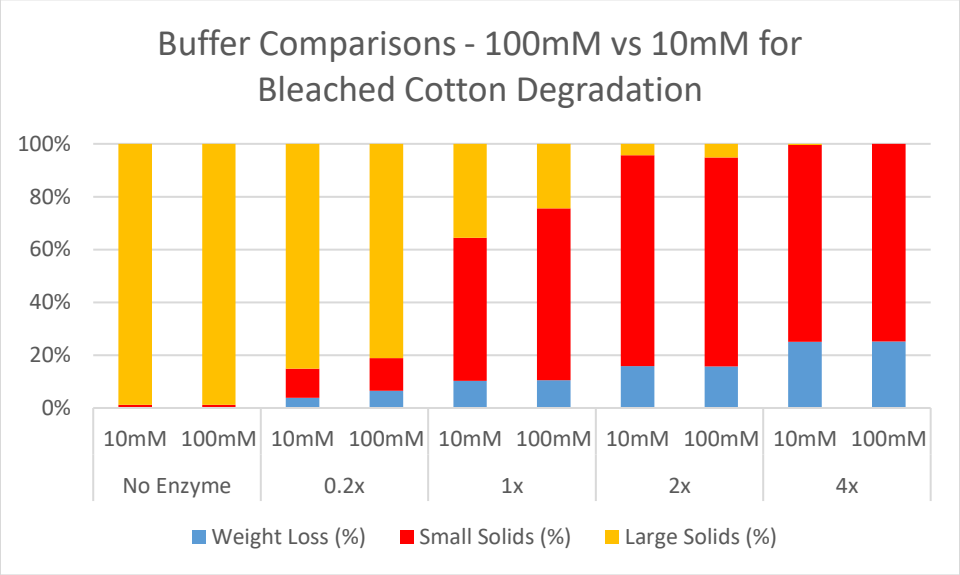


Figure 4.2.2. Average bleached cotton degradation at different enzyme doses, with comparisons between buffer concentrations.

Table 4.2.2. Average bleached cotton degradation across varying enzyme doses and different buffer concentrations.

Enzyme Treatment	Buffer Concentration (mM)	Weight Loss (%)	Small Solids (%)	Large Solids (%)
No Enzyme	10	0.12	1.01	99.08
	100	0.00	1.14	99.75
0.2x	10	3.97	11.23	86.92
	100	6.66	12.60	82.77
1x	10	9.97	52.47	34.55
	100	10.34	64.11	24.09
2x	10	15.76	79.68	4.28
	100	15.63	78.76	5.16
4x	10	24.92	73.99	0.38
	100	25.12	74.88	0.00

Despite the comparable degradation results between buffer concentrations at higher enzyme doses, there is an interest to understand the decrease observed at the lower enzyme dosages. As discussed earlier, the standard deviation of the gravimetric results was highest in the lower enzyme doses for UC due to inconsistent mechanical agitation during treatment. The same variation is not observed in samples with higher enzyme dosages because they incur more interactions between enzyme and fabric, able to fully degrade the fabric sample consistently despite the varied mechanical action between samples. So, it seems to be that a sufficiently high enzyme dose that has been shown to reduce variation in samples is also effective to reduce effects of buffer concentration.

4.2.3 Delta Cotton versus UC

Delta cotton (DC), being a commercial waste textile, had an unknown history of chemical treatment. It was assumed that because this fabric came from a standard white cotton t-shirt, it was simply bleached cotton. Given this assumption, the main difference between DC and the simulated bleached cotton (UC) is the fabric density, which was shown to be less for DC than UC (Table 4.2.3.1). After the same enzymatic treatment and buffer concentration, the two fabrics showed different levels of degradation at the same dosages (Figures 4.2.1 and 4.2.3/Table 4.2.1 and 4.2.3.2). For example, at a 1x dose, DC showed 12% weight loss and 8.2% large solids remaining whereas UC showed 10% weight loss and 24% large solids remaining when run at the same conditions. This indicates that fabric density played a role in the efficiency of fiber separation.

Table 4.2.3.1. Courses and wales per inch of each fabric type.

Fabric	CPI	WPI
Delta Cotton	40	27
Untreated Cotton	53	38
50/50 Polyester/Cotton	45	30
Blue Dyed Cotton	53	39
Red Dyed Cotton	55	40
Durable Press Finished Cotton	49	35
55/45 Modacrylic/Cotton	35	30

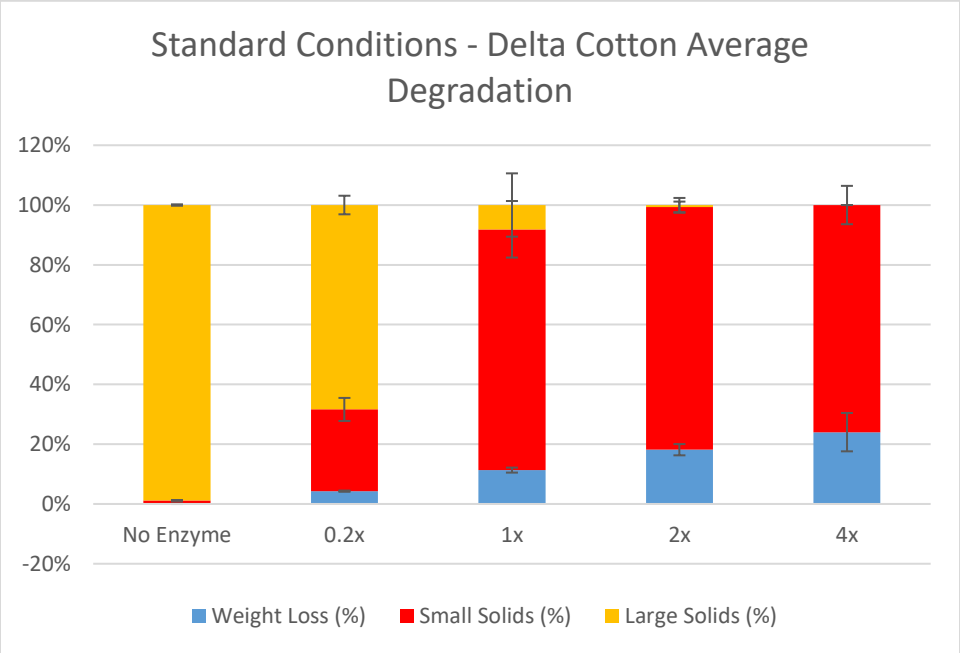


Figure 4.2.3. Average degradation of delta cotton t-shirt fabric using CTec2 at varying enzyme doses.

Table 4.2.3.2. Average degradation of delta cotton t-shirt at varying enzyme doses at standard conditions.

Enzyme Treatment	Weight Loss (%)	Small Solids (%)	Slurry (%)	Large Solids (%)
No Enzyme	0.08	1.11	1.17	98.85
0.2x	4.31	27.52	31.19	68.81
1x	11.52	81.91	91.74	8.25
2x	18.32	81.86	99.28	0.72
4x	24.02	75.98	100.00	0.00

The added space in between yarns in the DC fabric structure allowed more bath and enzyme penetration into the fabric structure, allowing for more interaction between cellulose fiber and enzyme during the incubation. There could also be a faster release of small solids from the knit after hydrolysis in the less dense fabric. During this treatment, the knit structure should influence the speed of small solid release, as the more entangled a hydrolyzed fiber fragment is within the knit, the longer it will take to "fall out" of the large solid piece, resulting in lesser size reduction of large solids to small solids. This would in turn reduce weight loss, as observed for the higher density UC fabric which had decreased conversion from fabric to soluble sugars.

4.2.4 Monocomponent Degradation of UC

In addition to Cellic® CTec2, an additional experimental multicomponent (NS 59150) and several monocomponent endoglucanases (NS 59149, NS 59102, NS 29077, and NS 59107) were also used to degrade bleached cotton fabric. Because the mechanism of endoglucanase enzymes is to cleave cellulose chains in the middle of the polymer (Figure 2.1.1.2a), significant production of soluble sugars was not expected for those degradation samples, meaning that significant weight loss was not expected. This idea was confirmed before degradation testing through reducing sugar assay where all monocomponent enzymes released glucose amounts below the detectable limit for

the assay (Figure 4.2.4.1 and Table 4.2.4.1). This was further supported in the degradation results where all of the monocomponents achieved weight loss of less than 7% (Table 4.2.4.2 and Figure 4.2.4.2). Though the degradation products in these weight loss portions were not investigated, it is possible that the soluble sugars are a combination of soluble oligosaccharides as well as glucose, but a high glucose content would be unexpected. Notably, the weight loss for NS 59149 and NS 59102 (7.0% and 6.0%) is higher than that for NS 29077 and NS 59107 (3.3% and 2.5%). This could be attributed to the formulation of the enzyme. While the two former were liquid formulations, the two latter were granular formulations, so it is possible that some particulate enzyme was collected and artificially increased the small solids yield in the case of the solid formulations, reducing the weight loss calculated. Otherwise, there could simply be a difference in the portion of soluble degradation products observed.

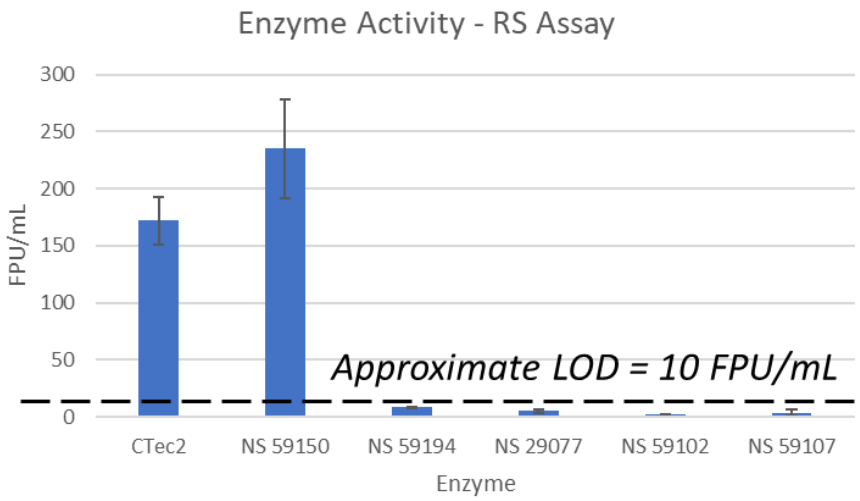


Figure 4.2.4.1. Reducing sugar assay results for Cellic® CTec2, an experimental multicomponent enzyme and four experimental endoglucanase monocomponents.

Table 4.2.4.1. Reducing sugar assay results for Cellic® CTec2, an experimental multicomponent enzyme and four experimental endoglucanase monocomponents.

		Multi	Liquid	Solid	Liquid	Solid
Enzyme Code	CTec2	NS 59150	NS 59149	NS 29077	NS 59102	NS 59107
Trial 1	184.44	229.38	8.93	6.97	1.75	3.17
Trial 2	183.66	280.91	7.34	3.61	2.04	6.21
Trial 3	147.45	195.16	8.19	4.98	1.72	1.57
Average	**171.85**	**235.15**	**8.15**	**5.19**	**1.84**	**3.65**
Std Dev	17.26	35.24	0.65	1.38	0.14	1.92

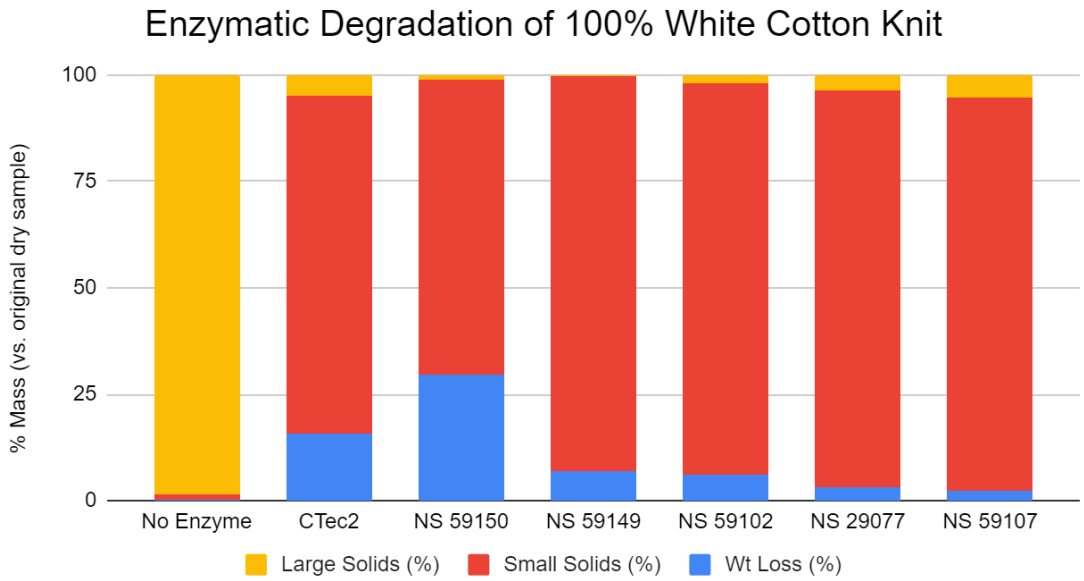


Figure 4.2.4.2. Comparison between 2x dose enzymatic degradation results of bleached cotton using two multicomponent mixtures (Cellic® CTec2 and NS 59150) and four endoglucanases (NS 59149, NS 59102, NS 29077, NS 59107).

Table 4.2.4.2. Multicomponent and monocomponent degradation results of bleached cotton at a 2x dose after 19 hours.

		Enzyme	Weight Loss (%)	Small Solids (%)	Large Solids (%)	Total Accounted (%)
		No Enzyme	0.10	1.21	98.69	100.00
Multicomponents		CTec2	15.7	79.5	4.8	100.0
		NS 59150	29.8	69.0	1.1	100.0
Monocomponents	Liquid	NS 59149	7.0	92.8	0.3	100.0
		NS 59102	6.0	92.2	1.8	100.0
	Solid	NS 29077	3.3	93.0	3.8	100.0
		NS 59107	2.5	92.2	5.3	100.0

The small solids yield made up almost the entirety of the gravimetric percentage for each of the endoglucanase samples (Figure 4.2.4.2). This was expected because of the endoglucanase mechanism, which is responsible for a major portion of the fabric disintegration in a multicomponent mixture. This emphasis on small solids production could be valuable as a degradation product isolation pathway, if small solids are the most desirable degradation product. Dosed at the same enzyme level as the Cellic® CTec2 samples, a higher yield of small solids can be obtained and, in some cases, can provide higher levels of large solid size reduction where NS 59149, NS 59102, and NS 29077 all had fewer large solids remaining on average than the Cellic® CTec2 samples (all less than 4%, where CTec2 had about 4.8% large solids remaining on average, see Table 4.2.4.1).

The tested multicomponent enzyme showed more efficient degradation of bleached cotton than Cellic® CTec2. Given their determined enzyme activities (Cellic® CTec2: 172 FPU/mL and NS 59150: 235 FPU/mL, see Table 4.2.4.1), it was understood that the glucose-releasing cellulases were more active in NS 59150 than Cellic® CTec2. Based on the degradation results, this was

confirmed in the weight loss data for each sample where on average, NS 59150 yielded a higher soluble sugar portion than Cellic® CTec2 (30% and 16%, respectively, see Table 4.2.4.2 and Figure 4.2.4.2). It was also observed that less large solids remained for the NS 59150 sample (1.1%) than the CTec2 sample (4.8%), achieving more efficient hydrolysis of cotton fabric. Despite this result, Cellic® CTec2 was used as the enzyme for all subsequent enzyme experiments due to its commercial status, which was more comparable to other published works and provided continuity between early and future samples tested during this research.

4.3 Chemical Obstacles

4.3.1 Blue Dyed Cotton Hydrolysis

When compared to UC hydrolysis data, results from UC fabrics dyed with Reactive Blue 19 (BC) and Reactive Red 198 (RC) offer quantitative evidence of the interference of dyes with degradation and the relative extent of that degradation based on dye molecule structure and properties. At a 4x enzyme dose, BC was fully degraded into a cellulosic slurry after 19 hours of enzymatic treatment (Figure 4.3.1.1). Because of reduced average weight loss in comparison to the UC average (18% and 25%, respectively, Tables 4.2.1 and 4.2.3), it could still be concluded that enzymatic hydrolysis is impeded by the presence of the dye. This is emphasized by the results from lower enzyme doses in comparing the large solids remaining. For example, at the 1x dose, BC fabric had just over 50% solids remaining whereas UC fabric had just over 30% solids remaining. This reduction in hydrolysis was expected because of the mechanism of dye-fiber interaction. Because reactive dyes covalently bond to a hydroxyl group on a glucan ring along the cellulose chain, the molecules are sitting along the polymer backbone, creating steric hinderance that the enzymes need to work around in order to interact with the glycosidic linkages between

rings. A cellulose sample with dye has fewer accessible reaction sites than an undyed sample (Figure 4.3.1.2) which reduces the speed at which hydrolysis reactions occur.

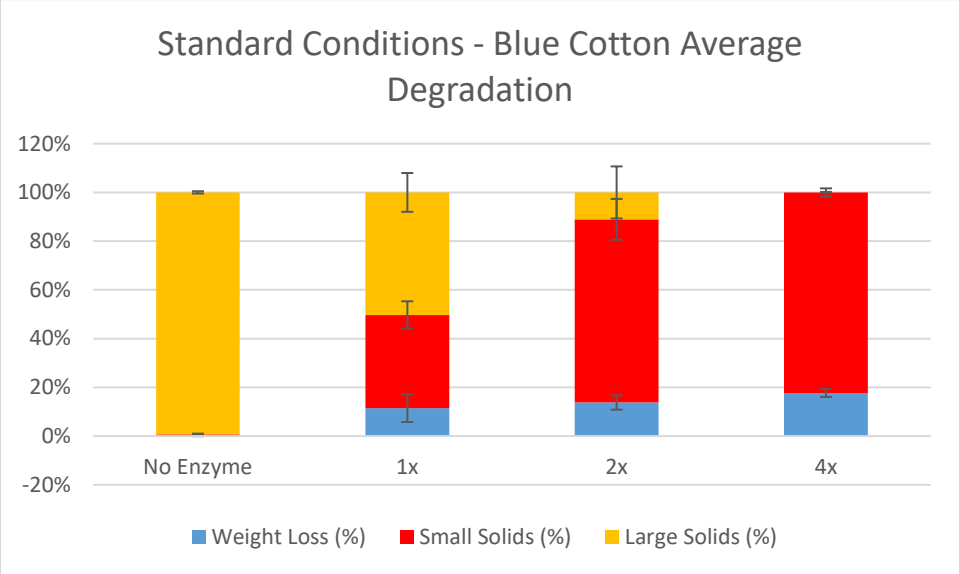


Figure 4.3.1.1. Average degradation of dyed blue 100% cotton fabric at varied enzyme concentrations.

Table 4.3.1. Average degradation of Reactive Blue 19 dyed cotton fabric at varied enzyme concentrations.

Enzyme Treatment	Weight Loss (%)	Small Solids (%)	Slurry (%)	Large Solids (%)
No Enzyme	0.01	0.76	0.89	99.11
1x	11.51	38.48	49.45	50.55
2x	13.92	75.92	88.85	11.15
4x	17.70	82.30	100.00	0.00

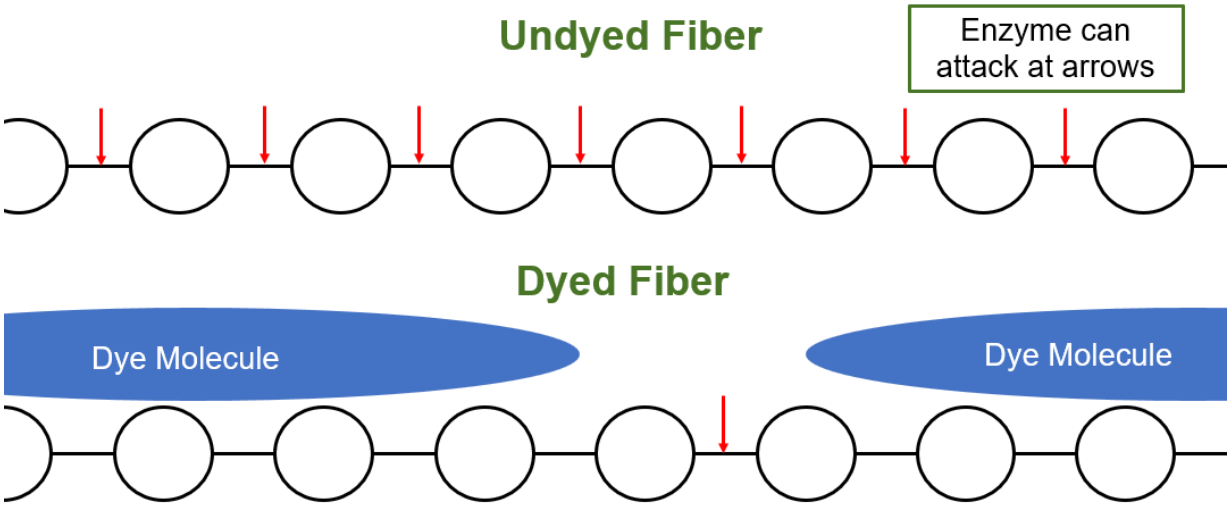


Figure 4.3.1.2. Schematic depicting how dye molecules interfere with enzymatic hydrolysis, where each "bead" is a glucose ring.

Notably, unlike the pattern observed with UC hydrolysis that showed minimal standard deviation for weight loss data, variation between samples of the same treatment was observed in all three gravimetric categories for BC hydrolysis data (Figure 4.3.1.1). This divergence from the pattern observed for undyed samples could be attributed to the steric hinderance of the dye molecule to the action of the different cellulase enzymes. So, the presence of the dye molecule not only influences the speed of fiber hydrolysis for fabric disintegration, but it also influences the speed of small-chain hydrolysis for weight loss, reducing its consistency across samples. This same trend in standard deviation was observed for other chemically modified cotton samples.

4.3.2 Red Dyed Cotton Hydrolysis

The impact of the dye molecule on fabric degradation is further emphasized with RC fabric hydrolysis, which was more impeded than BC fabric. After 19 hours of treatment at a 4x enzyme dose, RC fabric had 45% large solids remaining unlike the blue and undyed fabrics which had no large solids at the same conditions. This major difference can be attributed to the chemical structure of the two dyes (Figure 4.3.2.1). Reactive Blue 19 is a monofunctional reactive dye, meaning it

has one functional group capable of covalently linking to cellulose during dye fixation. Reactive Red 198 is a much larger bifunctional reactive dye, having two sites where it could covalently bond to cellulose during fixation. Previous studies reported that crosslinking of cellulose by bifunctional reactive dye led to reduced hydrolysis [30]. This crosslinking inhibits the mobility of the enzyme between chains and the independent mobility of the cellulose chains inside of a fiber, which would both decrease the frequency of enzymatic attack on the polymer backbone.

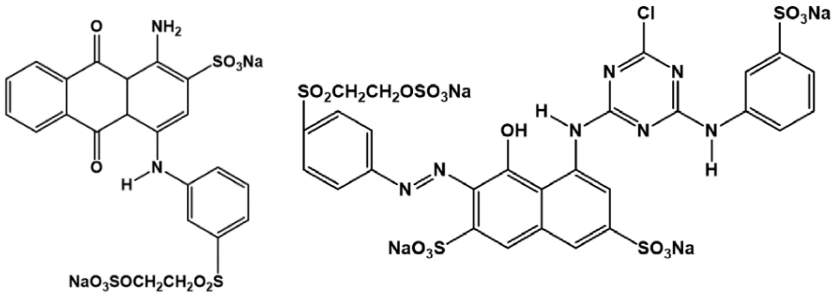


Figure 4.3.2.1 Chemical structures of Reactive Blue 19 (RB 19) and Reactive Red 198 (RR 198).

To push the RC fabric to fully convert into a slurry, further testing was conducted. Preliminary tests showed that even after extending incubation with a 4x enzyme dose up to 48 hours, 25% large solids remained undegraded (Table 4.3.2, Figure 4.3.2.2). This same test was later conducted for an even longer time, with full conversion to slurry of the red fabric only being achieved in the sample incubated for 96 hours (Figure 4.3.2.3). In response to this slow conversion, a new method was developed to shorten the incubation period required, therefore reducing the energy costs associated with heating and machine use. This new method was nominally called a double treatment. To start this new method, a standard 19-hour treatment was conducted at a controlled enzyme dose. Next, any slurry components were removed and collected through solid/liquid separation (bath and small solids). The collected large solids were then treated for a second 19-hour period at the same enzyme dose as the first, after which any final degradation

products were collected for analysis. Through this procedure, RC was able to be fully hydrolyzed into a slurry after 38 hours with a 4x enzyme dose. This procedure reduced the incubation time required by 60% but doubled the enzyme and buffer input, so the practical use of it would need to be investigated through cost analysis to determine if reducing enzyme input or incubation time is more financially beneficial. Regardless of method, the enzyme is able to overcome the obstacle of the bifunctional dye molecule with sufficient time and enzyme dosage, which is a positive indication for the future of this research and its relevance to textile circularity.

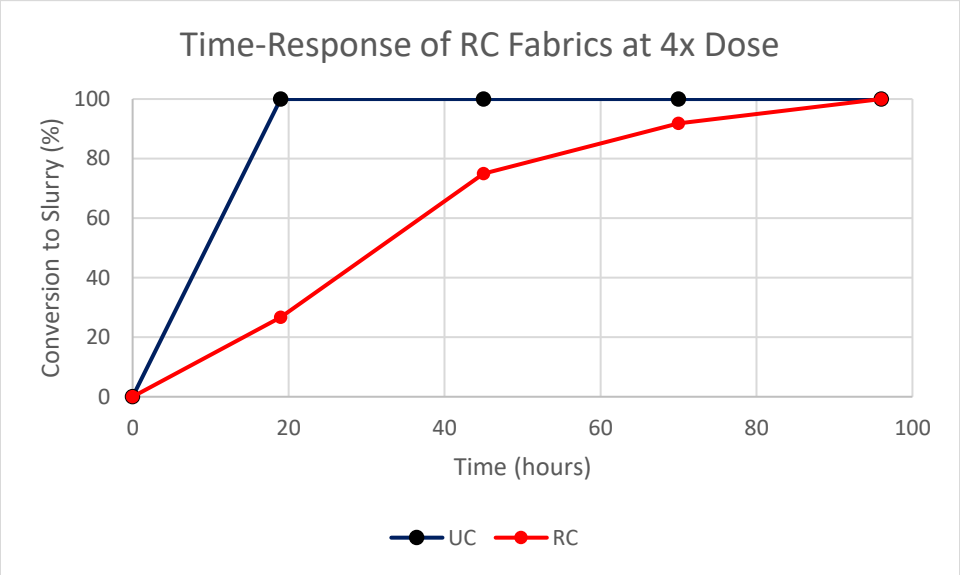


Figure 4.3.2.2. Conversion of red dyed cotton (RC) to slurry after 4x enzyme treatment at extended incubation lengths compared to the undyed cotton (UC).

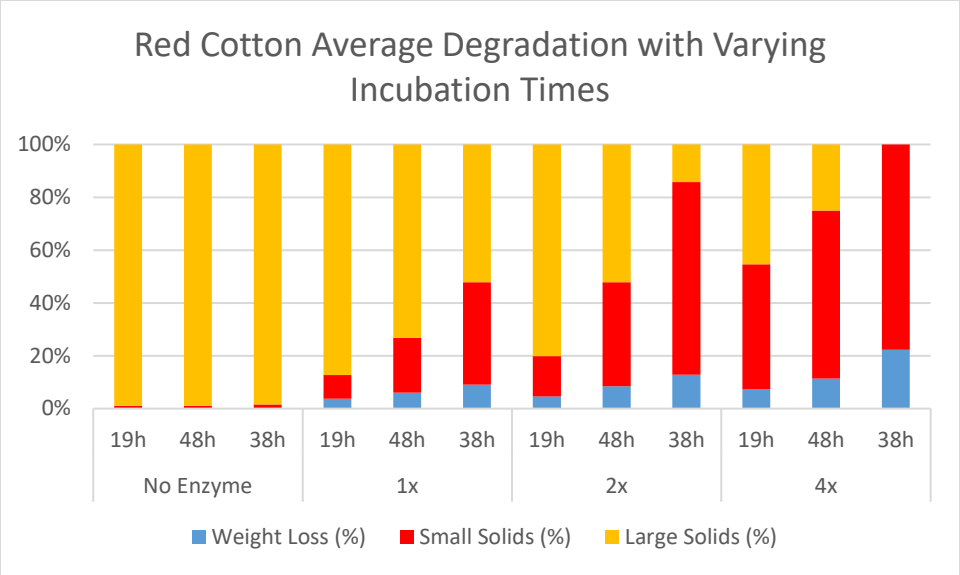


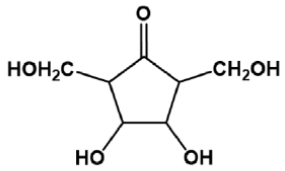
Figure 4.3.2.3. Average degradation of red cotton across different enzyme concentrations, comparing different incubation periods. 19h and 48h represent one enzymatic treatment, where 38h represents two enzymatic treatments (2 separate 19h treatments of the same fabric)

Table 4.3.2. Average degradation of red cotton at different enzyme concentrations and incubation times. Note: 38h of treatment is reflective of two enzymatic treatments (2 separate 19h treatments) whereas the 19 and 48h treatments are single enzymatic treatments.

Enzyme Treatment	Time	Weight Loss (%)	Small Solids (%)	Large Solids (%)
No Enzyme	19h	0.00	1.03	99.15
	48h	0.00	1.03	99.89
	38h	0.00	1.57	98.52
1x	19h	3.76	9.00	87.24
	48h	6.10	20.63	73.27
	38h	8.36	35.72	48.08
2x	19h	4.60	15.21	80.20
	48h	8.50	39.38	52.12
	38h	12.48	71.22	13.95
4x	19h	7.22	47.39	45.38
	48h	11.43	63.62	24.96
	38h	22.27	77.74	0.00

4.3.3 Durable Press Finished Cotton

The durable press finish was selected as one of the chemical obstacles because it was expected to be one of the most difficult common chemical finishes on apparel textile waste to overcome by enzymatic hydrolysis. Wrinkles are caused in cotton fabric during laundering because of hydrogen bond disruption which allows polymers and fibers to move independently of each other and resettle in new positions during drying. Any displacement during the reformation of those hydrogen bonds is what causes wrinkles. To overcome this for wrinkle resistant fabrics, a crosslinking agent (here, DMDHEU, Figure 4.3.3.1) is added to textiles to covalently link fibers and polymers together, preventing any slippage during washing. After treatment of the durable press finished, 100% cotton fabric for 19 hours with a 4x enzyme dose, less than 10% degradation was achieved (Figure 4.3.3.2, Table 4.3.3.1). Like the incidental crosslinking that occurred with the bifunctionally dyed fabric, this intentional and substantial amount of crosslinking significantly reduced the ability of the enzyme to reach the polymer backbone and carry out its hydrolytic action (Figure 4.3.3.3).



DMDHEU

Figure 4.3.3.1 Chemical structure of DMDHEU molecule

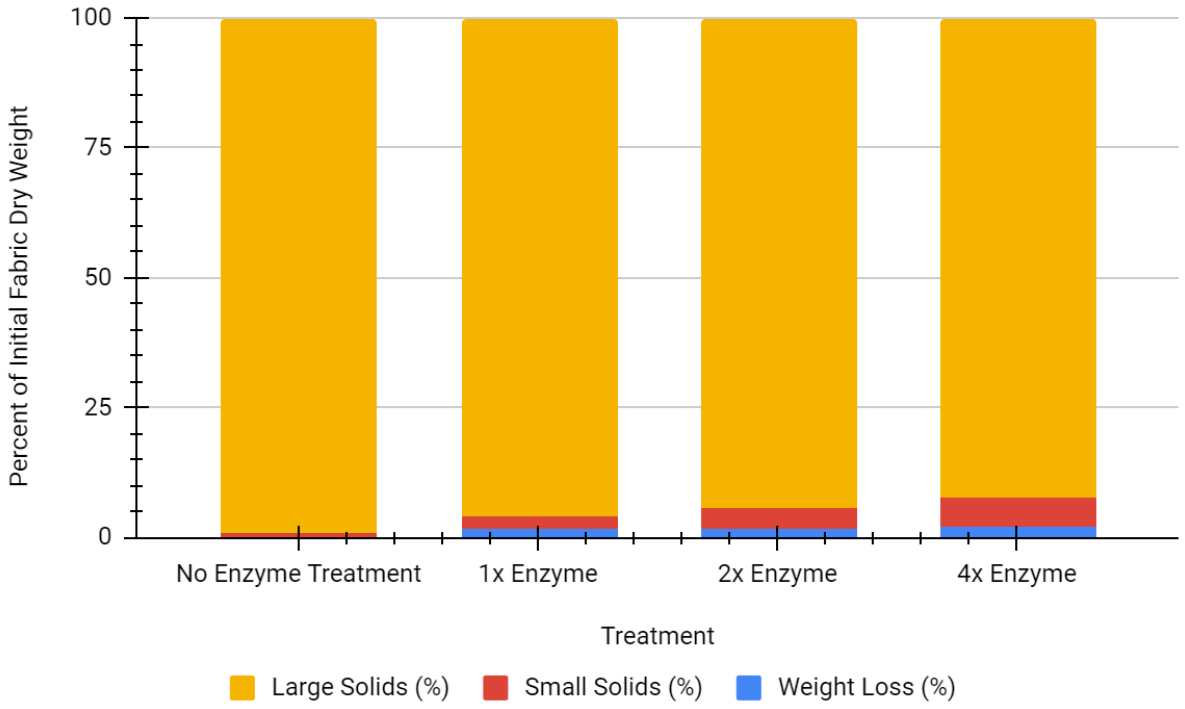


Figure 4.3.3.2. Average degradation of a durable press finished fabric at standard conditions across varying enzyme doses.

Table 4.3.3.1. Average degradation of durable press finished 100% cotton fabric after 19h at different enzyme doses.

Treatment	Weight Loss (%)	Small Solids (%)	Large Solids (%)
No Enzyme	0.05	0.63	99.32
1x	1.81	2.22	95.97
2x	1.80	3.87	94.32
4x	2.06	5.53	92.42

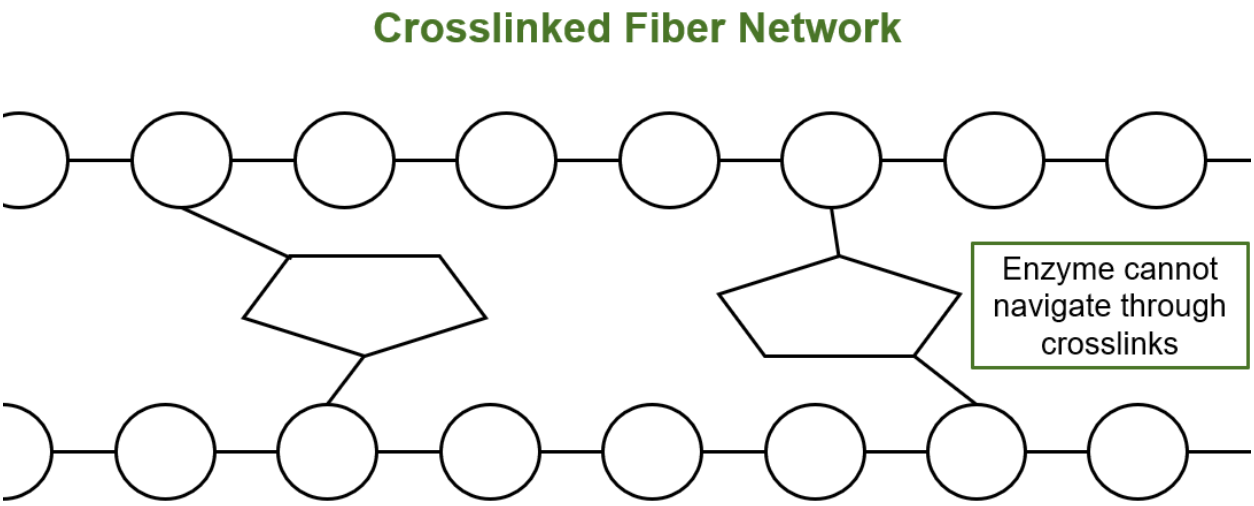


Figure 4.3.3.3. Schematic representation of durable press crosslinking chemistry and how it prevents enzymatic degradation of fibers.

Because enzymatic degradation alone was insufficient for breaking down the crosslinked cellulose quickly, chemical pretreatments that could mitigate or eliminate the effect of the crosslinker were investigated. Applying a cold alkali pretreatment was expected to cause fiber swelling, increasing distance between cellulose chains to allow more enzyme mobility through the polymer network and decreasing crystallinity, which would increase the amount of fiber that would be susceptible to enzymatic attack [36]. After this pretreatment and a 4x enzyme treatment, conversion to slurry was doubled to about 20% (Table 4.3.3.2). Despite the improved results, it was concluded that the cold alkali was not able to induce a high extent of degradation, so the addition of an acid pretreatment step was tested which was previously shown to be an effective DMDHEU crosslink hydrolysis method [62]. A low acid concentration was used to prevent significant cellulose damage or degradation during chemical treatment. The acid hydrolyzed the bonds between the crosslinker and cellulose, increasing chain mobility within fibers and allowing more access of the enzyme to the individual polymers. The cold alkali treatment followed acid hydrolysis to remove any fully hydrolyzed DMDHEU molecules as well as increasing fiber

swelling. After this sequential chemical treatment and a 4x enzyme dose, degradation of the DP fabric was improved to about 60% (Figure 4.3.3.4). Given this positive result, the same double treatment applied to RC was used for the remaining large solids, which was able to push the fabric to full conversion into a slurry (less than 1.5% large solids remaining, Table 4.3.3.3) after less than two days of total processing. Though chemical pretreatments of extreme pH are not the most desirable, especially for sustainable development, the application of them to overcome major obstacles to textile recycling, namely chemical additions onto fabrics, cannot be overstated.

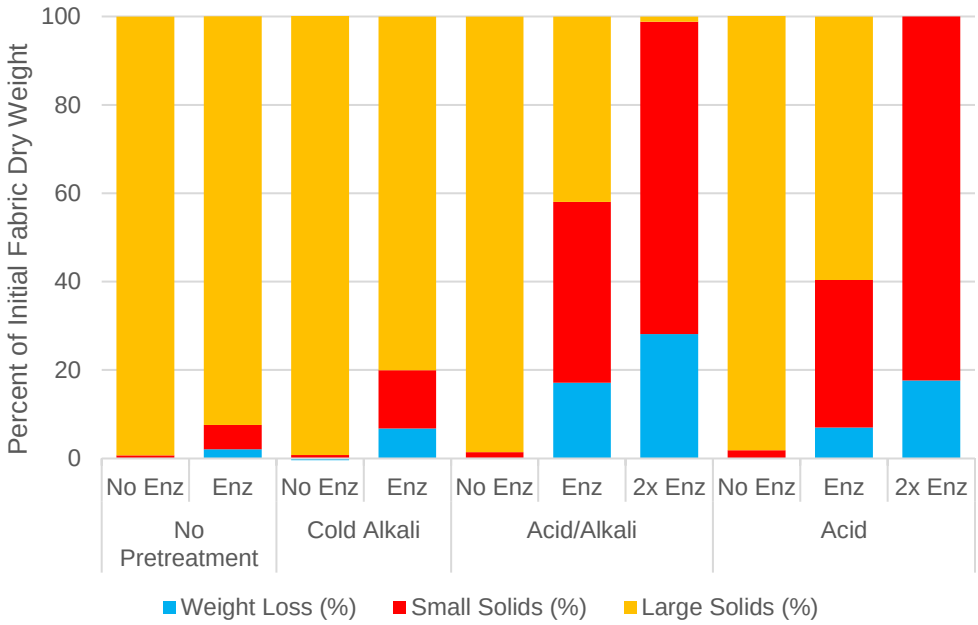


Figure 4.3.3.4. Average degradation of durable press finished 100% cotton fabric treated with either no enzyme (No Enz) or a single 4x dose (Enz) or two separate 4x dose treatments (2x Enz) after the specified pretreatment.

Table 4.3.3.2. Average degradation of durable press finished 100% cotton fabric after pretreatment followed by no enzyme (No Enz), one 4x dose enzymatic treatment (Enz), and two 4x dose enzymatic treatments (2x Enz).

Sample	Enz Treatment	Weight Loss (%)	Small Solids (%)	Large Solids (%)
No Pretreatment	No Enz	0.05	0.63	99.32
	Enz	2.06	5.53	92.42
Cold Alkali	No Enz	-0.4	0.76	99.64
	Enz	6.79	13.19	80.02
Acid/Alkali	No Enz	0.16	1.26	98.58
	Enz	17.17	40.89	41.94
	2x Enz	28.12	70.66	1.22
Acid	No Enz	-0.12	1.89	98.23
	Enz	7.03	33.33	59.64
	2x Enz	17.66	82.34	0

Because alkali pretreatment was not necessary for previous fabric types, it was useful to test the impact that an acid treatment alone would have on degradation of the durable press finished fabric. This would theoretically leave any loose DMDHEU molecules on the surface of the fabric during enzymatic treatment, which could negatively affect enzymatic hydrolysis. Because of the potential for the acid to degrade cotton, fabric weight loss during acid treatment was measured and shown to be 3.56% (Table 4.3.3.3). Some of this weight loss could be attributed to the removal of DMDHEU molecules during washing with DI water to neutralize the fabric. Otherwise, there could have been some fiber damage or degradation because of the acid treatment. All chemically pretreated enzymatic degradation samples were still measured to a starting weight of 1g after pretreatment, meaning the degradation results pictured in Figure 4.3.3.4 are representative of only enzymatic degradation and do not account for any weight loss from acid treatment.

Table 4.3.3.3. Measured weight loss during acid pretreatment.

Acid Treatment			
Initial Fabric Weight (g)	20.9	Final Fabric Weight (g)	20.0
Average Moisture Content (%)	5.90	Average Moisture Content (%)	5.11
Corrected Dry Weight (g)	19.7	Corrected Dry Weight (g)	18.9
Weight Loss (%)		**3.56**	

After acid pretreatment followed by enzymatic treatment at a 4x dose, about 40% degradation was achieved (Table 4.3.3.2). A double treatment was carried out as a comparison to the acid/alkali pretreated fabric and surprisingly yielded no large solids for the acid treated fabrics (Figure 4.3.3.4). Because the degradation after the first treatment was smaller than the acid/alkali treated samples, it was unexpected that the acid treated samples would fully degrade. Unlike the acid/alkali treated samples which had a small percentage of large solids remaining, none of the acid treated samples had large solids remaining after the second enzymatic treatment.

4.3.4 Polyester/Cotton Blend Hydrolysis

Fabric blends are typically made by blending fibers during yarn construction before being knit or woven into a fabric, which creates a physical obstacle for enzymatic hydrolysis of cotton fibers. Because cellulase enzymes are selective in their action, they only target the cellulosic portion of a blend. Therefore, the presence of intimately blended synthetic fibers acts as a large physical block to fabric structure disintegration. In other words, some cotton fibers can be “protected” from enzymatic degradation (and the mechanical agitation that promotes degradation) by being shielded within the synthetic fiber fabric structure.

When 100% cotton was enzymatically degraded by the 2mag procedure, the entire fabric structure gradually disintegrated from the edges inward into a slurry of small solid fragments and soluble compounds. In a blended fabric, the synthetic fibers retain their shape and fabric structure,

blocking the release of degraded cotton fragments, which remain entangled in the large solids until they degrade to a sufficiently small particle size to fall out of the structure. Vigorous washing after enzymatic hydrolysis is therefore very important for removing any degraded fragments that are "stuck" in the twisted synthetic yarns of the residual large solid fabric pieces. When measuring extent of degradation by gravimetric analysis, 50% large solids remaining for a 50/50 polyester/cotton fabric would indicate full conversion of the cotton portion of the blend to slurry, assuming efficient separation of all cotton fragments by enzymatic treatment and washing, such that all remaining large solids are assumed to be polyester.

After treating an undyed 50/50 polyester/cotton blend at standard 19-hour conditions, a high extent removal of cotton was observed at the highest enzyme dose (Figure 4.3.4). However, some residual cotton remained in the large solids fraction. Microscopic observations indicated the residual cotton was "buried" and entangled within the intact twisted yarn structure of the undegraded polyester (Figure 4.6.1.2). Thus, the physical entanglement of cotton fibers with polyester fibers inhibited enzyme hydrolysis as expected, protecting some cotton fibers from the physical stresses of the beating stir bars that would otherwise enhance enzyme action. After a standard double treatment, both the 2x and 4x dose samples on average had around 53% large solids remaining (Table 4.3.4). Despite not achieving 50% large solids remaining, these samples were considered fully separated.

The figure of ~53% residual large solids was repeatedly observed for PC samples after a 4x double treatment and in many samples after a 2x double treatment. The same ~53% residual large solids value was also observed for the 4x dose after an extended single treatment of 24 hours (Table 4.3.4). Because of this result, somewhat extending the treatment time could be more energy efficient than carrying out a double enzyme treatment. A future research question is whether the

small amount of residual cotton could be removed through a separate melting and filtering step if needed.

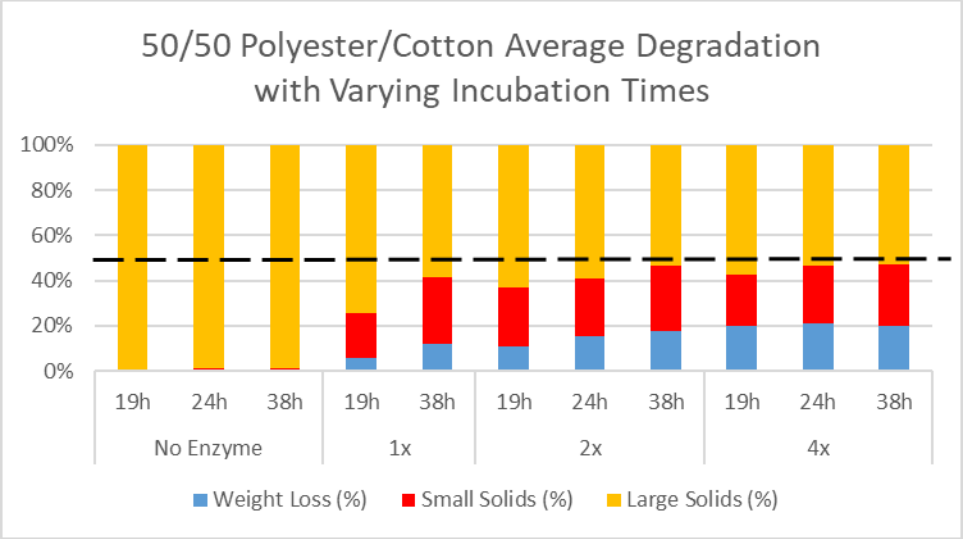


Figure 4.3.4. Average degradation of 50/50 polyester/cotton undyed fabric across different enzyme doses and incubation times. 19 and 24h treatments are one single treatment and 38h is two separate 19h enzymatic treatments. The dashed line shows maximum degradation at 50% because that is the fabric weight attributed to polyester fiber.

Table 4.3.4. Average degradation of 50/50 polyester/cotton fabric at different incubation times and enzyme doses. The 38h treatment time is indicative of two separate 19h treatments on the same fabric.

Enzyme Treatment	Time	Weight Loss (%)	Small Solids (%)	Large Solids (%)
No Enzyme	19h	0.00	0.57	99.84
	24h	0.00	0.94	99.97
	38h	0.00	1.04	99.19
1x	19h	5.48	20.32	74.20
	38h	11.52	29.14	57.11
2x	19h	10.74	26.30	62.96
	24h	15.60	25.26	59.14
	38h	17.57	28.77	53.35
4x	19h	19.60	22.82	57.58
	24h	20.95	25.45	53.61
	38h	19.92	27.23	52.57

4.3.5 Time Response of Fabrics

Enzymatic degradation experiments were performed on several 100% cotton fabrics and a 50/50 polyester/cotton fabric to gather information about the kinetic response of each fabric to enzymatic hydrolysis. The fabrics tested were UC, BC, RC, acid-pretreated DP, and PC (Figure 4.3.5). Each fabric had one sample dosed with a 4x enzyme dose that was allowed to run for either 19, 45, 70, or 96 hours according to the standard run conditions. While the 100% cotton fabrics are plotted by their conversion to slurry after a certain incubation time, the PC fabric is plotted as cotton removal based on the initial starting weight of cotton being 50% of the fabric weight.

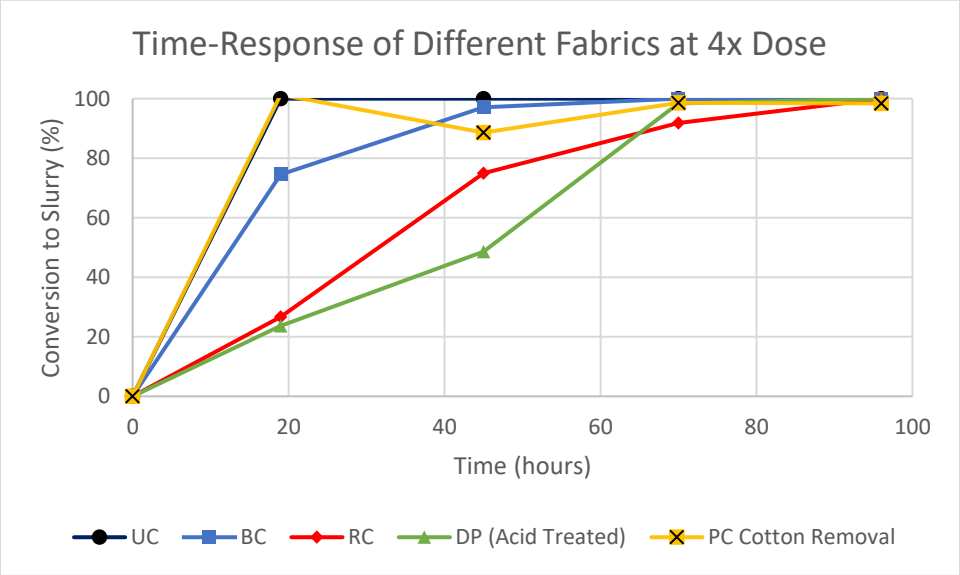


Figure 4.3.5. Comparison of the rates of conversion to slurry at a single 4x dose between fabrics with different levels of chemical or physical obstacles including untreated cotton (UC), blue dyed cotton (BC), red dyed cotton (RC), acid-pretreated durable press finished cotton (DP), and cotton removal from a 50/50 PET/cotton blend (PC Cotton Removal).

It was observed in early experimentation for 2mag machine consistency testing that fabric degradation was negatively impacted by sample positioning (see Appendix A-6). Samples surrounded on all sides by other actively mixing samples consistently showed less degradation than those that had an “exposed” side or were in one of the outer sample slots. Because of this,

experiments with a maximum of 12 samples were commonly run to minimize magnetic interference between samples. The time-response study used all 15 sample slots for the first 19 hours of incubation, which could explain some variation from average sample data or expected sample degradation.

Notably, each fabric and incubation length pair was only represented by a single sample, which means sample variation based on mechanical agitation was not accounted for in this trial. For example, PC45 (that is, PC fabric processed after 45 hours of enzymatic treatment) showed less cotton removal than PC19 (Figure 4.3.5), which is attributed to inconsistent mechanical agitation between samples. Given previous PC experiments, it was concluded that the PC19 sample showed abnormally high degradation. Average large solids remaining after a 19-hour PC enzymatic treatment was 58% (Table 4.3.5) when including all samples tested at this condition, equivalent to about 84% cotton removal. The PC19 sample during the time-response study gravimetrically showed 101% cotton removal (Table 4.3.5), indicating more effective and aggressive mixing on the fabric and possibly a higher amount of microfiber shedding from the polyester component because of it, leading to a value of greater than 100% theoretical removal. The PC19 sample was in a corner slot in the 2mag, which could help explain this increased degradation. The average value of 84% cotton removal for 19-hour samples makes more sense with the PC45 sample, which showed almost 89% removal, a slight improvement. Notably, even after 96 hours of treatment, the PC fabric did not gravimetrically show 100% cotton removal, with both PC70 and PC96 reaching around 99% removal according to gravimetric results. This result could suggest either that some small amount of cotton will always remain trapped in the fabric structure or that the fabric composition is slightly varied from the declared blend ratio, which could

perhaps have more polyester than claimed. To test the latter theory, other methods of blend separation (like dissolving cotton or polyester using chemical solvents) could be investigated.

Table 4.3.5. Conversion of different fabrics to slurry over time.

Time (h)	0	19	45	70	96
UC	0	100	100	100	100
BC	0	74.48	97.14	100	100
RC	0	26.63	74.99	91.8	100
DP (Acid treated)	0	23.71	48.57	98.41	100
PC (Cotton Removal)	0	101.34	88.57	98.63	98.37

UC fabric was shown to be 100% converted into a slurry after all incubation times, which was expected from previous UC experiments. The incubation times beyond 19 hours were tested despite this expected 19-hour result to generate small solids samples for degradation product characterization (see Section 4.5). For both the 19- and 45-hour samples, BC fabric was not fully converted into a slurry. This behavior was a significant divergence from the average BC degradation results, which consistently showed full conversion to BC slurry after 19 hours at a 4x dose (Figure 4.3.1.1). This is primarily attributed to inconsistent sample agitation which could have been impacted by the use of every sample slot in the 2mag, where the BC45 sample specifically was surrounded on each side by other samples during its incubation.

Notably, the reduced degradation for BC fabrics could also be explained by reduced enzyme activity, which is known to decrease as the enzyme samples becomes less “fresh”. A reducing sugar assay conducted shortly after the time-response study yielded an activity result of 140 FPU/mL, which is slightly lower than the nominal 172 FPU/mL (Table 4.2.4.1) established through earlier assays for a fresh sample of the CTec2 formulation. The lower enzyme activity could have induced less efficient hydrolysis of the fabrics. Supporting this explanation, both RC and DP fabrics showed less degradation after 19 hours than their average values from previous

samples. RC typically reached around 45% conversion to slurry after 19 hours (Table 4.3.2), but in this trial only reached about 26% (Table 4.3.5), and acid pretreated DP previously showed about 40% degradation after 19 hours (Table 4.3.3.2) while the sample in this trial reached about 24% (Table 4.3.5). The same activity explanation is not supported by the PC19 data, which showed improved degradation to average values after 19 hours. So, the observed lower or higher extents of degradation throughout this study could be due to a combination of multiple factors, where sample slot and resultant mechanical agitation as well as lower enzyme activity played a role in noticeable variations from average results.

RC and acid-pretreated DP fabrics were known to require a double treatment for full conversion of fabric to slurry, so this time response study was telling for each of their general kinetic responses to enzymatic treatment, especially in comparison to each other. RC fabric showed faster initial degradation, reaching almost 75% by 45 hours, whereas DP was slower in this range, not quite reaching 50% degradation after 45 hours. By 70 hours, the rate of degradation for DP had surpassed RC (Figure 4.3.5), which slowed down as the incubation time went on. At 70 hours, DP showed 99% degradation whereas RC showed 92% (Table 4.3.5). Similar behavior was observed previously during double treatment experiments, where RC showed more degradation after 19 hours than the acid-pretreated DP samples on average, but both showed consistent full degradation after the second treatment.

This phenomena could be explained by the respective chemistries of each fabric. Because RC fabric was concluded to be crosslinked by the bifunctional reactive dye based on degradation results, it seems likely that the enzyme is attacking non-crosslinked amorphous areas of fibers first, leaving a more stubborn large solid fraction with a higher concentration of crosslinks over time. This differs from DP fabric which was assumed to have even crosslinking across the whole fabric

after DMDHEU application and theoretically even crosslink hydrolysis during acid pretreatment. For that reason, it is possible that the acid-pretreated DP substrate is more uniform in terms of its crosslinked status than the RC substrate. Because enzyme concentration doesn't change during the incubation and large solids weight decreases, the ratio of enzyme-to-fabric pieces increases over time. In a practical sense, the enzyme dose "increases" as incubation time increases. For DP fabric, this increased enzyme-to-fabric ratio could have caused for an increase in degradation rate over time, which could explain the large jump between DP45 and DP70 degradation (Figure 4.3.5). For RC fabric, this increase in dose was possibly counterbalanced by the increased crosslink "concentration" in the remaining large solid pieces. By 96 hours, both samples were fully degraded, which was a major extension from the 38 hours used for a double treatment. This relationship between extended time versus additional enzyme would have major implications for cost-efficiency of this process in practice and would need to be explored based on relevant cost of energy and enzyme inputs.

4.3.6 Commercial Fabrics

4.3.6.1 KK Fabric Hydrolysis

The term "combination fabric" herein describes fabrics that contain more than one of the previously described obstacles to enzymatic hydrolysis, like a fiber blend fabric that is also dyed. One combination fabric tested was a commercial 55/45 modacrylic/cotton khaki dyed fabric (KK). With this blend composition, complete removal of cotton would leave 55% large solids as cleaned modacrylic. Notably, this sample was a double knit or "interlock" knit, which differed from the simulated waste samples tested previously (all single jersey knit). A double knit fabric is typically thicker than a jersey knit, and characteristically does not curl the way jersey knit fabrics do, which could have an impact on the degradation treatment results. The presence of the khaki dye colorant

was expected to interfere with hydrolysis, but the specific dye(s) applied were not known as the commercial fabric was received colored.

At a 4x enzyme dose, 58% large solids were recovered after the standard 19-hour hydrolysis treatment, meaning 42% of the initial fabric was converted to slurry by enzymatic treatment (Figure 4.3.6.1). While this was gravimetrically close to complete fiber separation (~93% cotton removal), some cotton fibers were still trapped in the twisted yarn structures. After a double treatment (38 hours total), all three tested enzyme doses achieved nearly complete cotton removal (56-57% large solids remaining, Table 4.3.6.1). Importantly, even though the 1x dose had a few percent cotton remaining (~96% cotton removal), results show that the dyed double knit KK fabric required less enzyme to fully remove cotton than the undyed jersey knit PC, which had significant cotton remaining after a 1x double treatment (~86% cotton removal, Figure 4.3.4).

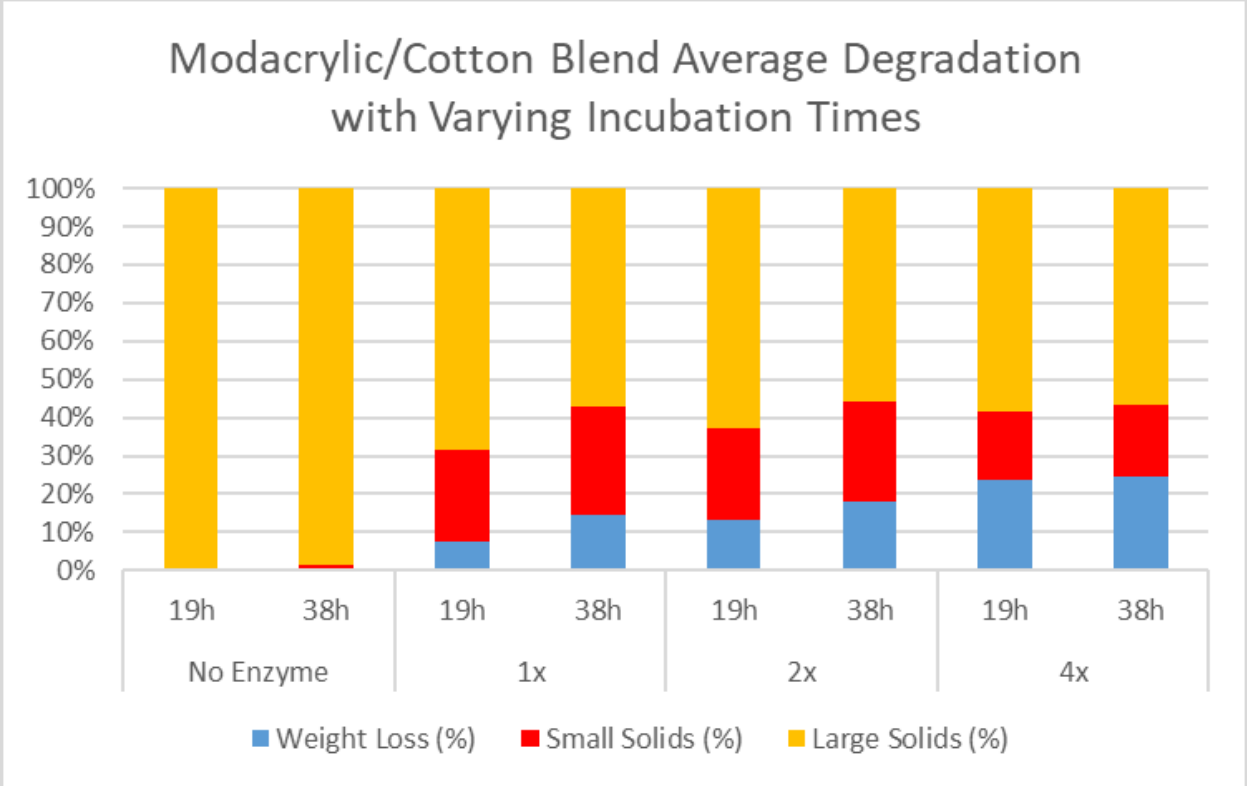


Figure 4.3.6.1. Degradation of 55/45 modacrylic/cotton khaki interlock knit fabric after either a 19 hour single treatment or a 38 hour double treatment.

Table 4.3.6.1. Gravimetric results from the degradation of a 55/45 modacrylic/cotton blend after the specified time with different enzyme doses.

Enzyme Treatment	Time	Weight Loss (%)	Small Solids (%)	Slurry (%)	Large Solids (%)
No Enzyme	19h	0.00	0.63	0.41	99.59
	38h	0.00	1.36	1.05	98.95
1x	19h	7.57	24.01	31.58	68.42
	38h	14.75	28.31	43.05	56.95
2x	19h	13.25	23.84	37.09	62.91
	38h	17.87	26.37	44.23	55.77
4x	19h	23.86	17.69	41.55	58.45
	38h	24.45	18.82	43.27	56.74

The favorable behavior of the double knit fabric construction is attributed to minimal fabric curling or folding of fabric swatches during enzymatic treatment. The recovered KK synthetic solids remained flat, which allowed for easier cotton fiber disentanglement during incubation and washing cycles. In contrast, the PC jersey knit fabrics became curled and folded during treatment, resulting in cotton fibers becoming entangled or trapped within the fabric folds and not washing out. Other physical properties of synthetic fibers like fiber diameter and stiffness or construction characteristics like yarn size and twist factor of the blended yarns, could contribute to the ease or difficulty of removing enzymatically degraded cotton fragments from synthetic/cotton blends. Measuring these aspects should be considered in future work.

4.3.6.1.1 Scale Up Testing

One large KK fabric piece (~300g) was enzymatically treated in a mini-jet dyeing machine for two separate 4x enzyme dose incubations, each for 24 hours, between which a solid/liquid

separation was performed as done previously in lab-scale double treatment experiments. A mini-jet dyeing machine was used because of its operating mechanism which allowed for both fabric and bath cycling throughout the treatments. This simultaneous cycling meant mechanical agitation was driven by bath movement through the fabric structure during incubation. Given the known importance of mechanical agitation in coordination with enzymatic degradation efficiency, that processing condition was a key point of interest during this scale-up trial.

After both enzyme treatments, about 21% fabric degradation was achieved (Table 4.3.6.1.1), which was much lower than the possible 45% degradation. To better describe the results, fabric degradation data was converted to theoretical cotton degradation (right of Figure 4.3.6.1.1) to show how much of the original cotton remained in the fabric after degradation, totaling about 53%. Of course, the weight loss of the original fabric could have included inherent synthetic fiber shedding on top of the induced cotton fiber hydrolysis, so the cotton degradation could be artificially high, but it is expected to be close to the true value.

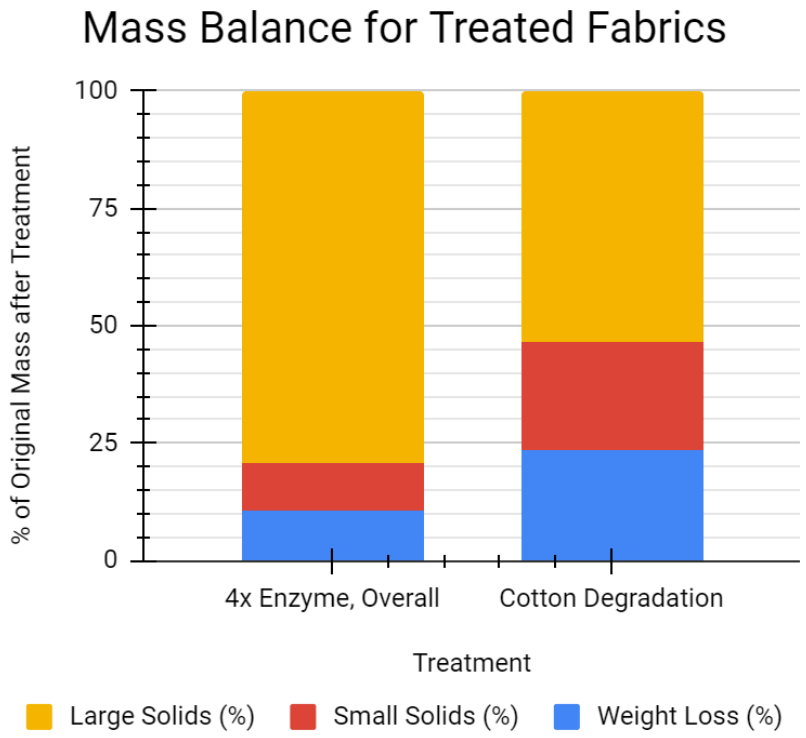


Figure 4.3.6.1.1. Results from a 300x scale-up trial conducted in a mini-jet dyer on 55/45 modacrylic/cotton khaki knit fabric after two 24 hour incubations.

Table 4.3.6.1.1. Gravimetric results from mini-jet dyer scale up trial after two 24 hour incubations with 55/45 modacrylic/cotton fabric.

Treatment	Weight Loss (%)	Small Solids (%)	Large Solids (%)
4x Enzyme, Overall	10.60	10.35	79.05
Cotton Degradation	23.56	23.01	53.44

A number of effects contributed to incomplete cotton removal during this trial. First, the jet dyer experienced processing complications overnight as the fabric became wrapped around the beam that controls fabric cycling. This entanglement prevented the fabric from cycling during the overnight period. The mechanical failure meant a large percentage of the consequently immobile fabric was not exposed to enzyme liquid during a significant portion of the incubation.

Furthermore, because this machine is designed to dye fabrics, which needs to be carried out without fabric damage, the level of mechanical action imparted by the jet was ultimately too gentle to enhance enzymatic fabric hydrolysis. Finally, previous lab-scale experiments showed that fabric swatch size influenced degradation speed where smaller swatches exhibited increased conversion to slurry (see Appendix A-1), so the degradation was likely impaired by the large fabric size as well.

Both large fabric size and mechanical agitation were impactful in the fabric washing step after enzymatic hydrolysis as well. In the 2mag methodology, a series of fabric washes (vigorous shaking in a bottle) aided in short fiber disentanglement and removal, resulting in cleaner synthetic fiber residuals. The reduced level of mechanical agitation in the jet dyer was not able to effectively remove degraded fibers from the fabric during washing steps. After several washes, the large fabric piece had visually apparent collections of degraded fibers remaining on its surface on top of being entangled in the fabric structure. This trial informed that aggressive mechanical agitation, fabric swatch size, and washing of synthetic solids are all key elements of enzymatic fiber separation methodology that need to be addressed for future scale-up trials.

4.3.6.2 NP Fabric Hydrolysis

A 55/45 synthetic/cotton blend fabric (NP, for navy pants) dyed to a deep navy-blue shade and finished with a durable press crosslinking agent (deemed as a “worst case scenario” fabric), was tested. The depth of shade of a fabric is controlled by the amount of dye on the fabric, so a darker shade would indicate that more dye molecules are present on the fabric, providing more obstruction than a fabric colored to a lighter shade with the same dye. The compounded obstacles were expected to significantly reduce the ability of this fabric to degrade. Interestingly, this commercial fabric was of a different construction than the jersey knit simulated waste fabrics, as

it was a twill-weave woven fabric, which could influence the cotton removal behavior. More detailed fabric construction aspects could be studied in future work.

Because the crosslinking finish alone was known to inhibit degradation, rather than testing at standard 2mag conditions, NP fabric was initially tested using the chemical pretreatment and double treatment enzymatic conditions that were previously successful for durable press 100% cotton fabric. Because of the prior success with the acid pretreatment alone, the same pretreatment and enzymatic conditions were used for this combination fabric, resulting in 78% large solids remaining (Table 4.3.6.2). According to the blend ratio of this fabric, 55% residual large solids would represent full cotton removal, so the acid pretreatment alone followed by a double enzymatic treatment was insufficient to deliver a high level of cotton degradation (Figure 4.3.6.2.1).

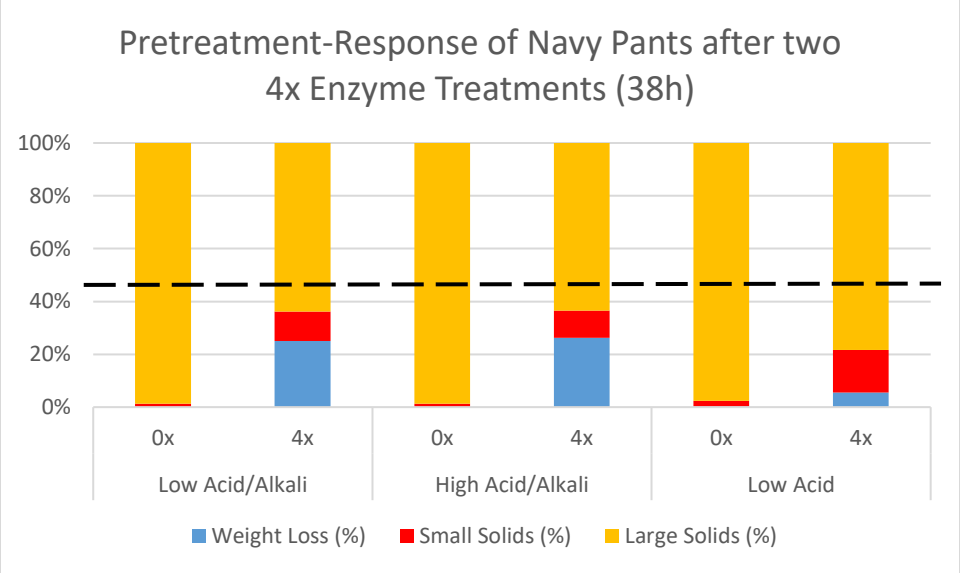


Figure 4.3.6.2.1. Overall degradation of navy pants fabric after listed pretreatment and two 19h enzyme treatments, where the dashed line shows the expected degradation at 45% because 55% of the fabric is synthetic.

Table 4.3.6.2. Summary of overall degradation of navy pants fabric after chemical pretreatment and two enzyme treatments.

Pretreatment	Dose	Weight Loss (%)	Small Solids (%)	Large Solids (%)
Low Acid/Alkali	0x	0.09	1.22	98.70
	4x	25.05	11.15	63.80
High Acid/Alkali	0x	0.13	1.20	99.10
	4x	26.24	10.36	63.40
Low Acid	0x	0.21	2.18	98.10
	4x	5.55	16.07	78.39

An additional trial incorporating the alkali pretreatment step to swell cotton fibers and help remove severed crosslinks from the fabric was performed. In this case, after sequential acid, alkali, and enzymatic double treatment at a 4x dose, the NP fabric had 64% large solids remaining (Table 4.3.6.2), which corresponds to about 80% cotton removal (Figure 4.3.5.6.2). In addition to disrupting crosslinking molecules, some dye molecules were also removed from the fabric during these pretreatments, though the fabric still retained its deep navy color. In an effort to sever more crosslinks and increase cellulose degradation, the acid concentration was modified from a 1% volume solution to a 5% solution during chemical pretreatment. A higher level of dye removal during the alkali step was visually observed after the adjusted pretreatment with higher acid concentration, suggesting a higher level of covalent bond disruption. Despite this indication, the degradation results were unchanged between the two procedures. After the adjusted “High Acid/Alkali” (Figure 4.3.6.2.1) treatment, 63% large solids were retained (Table 4.3.6.2), which was similar to the result from the “Low Acid/Alkali” treatment performed under the same conditions as the DP fabric. These results suggest there is another unknown obstacle (potentially the fabric structure) in this fabric that is preventing full cotton removal that was unaccounted for by the chemo-enzymatic procedure. Despite this, the high level of cotton removal from this worst-

case scenario fabric type is a very positive result for the efficacy of this methodology in the textile recycling space.

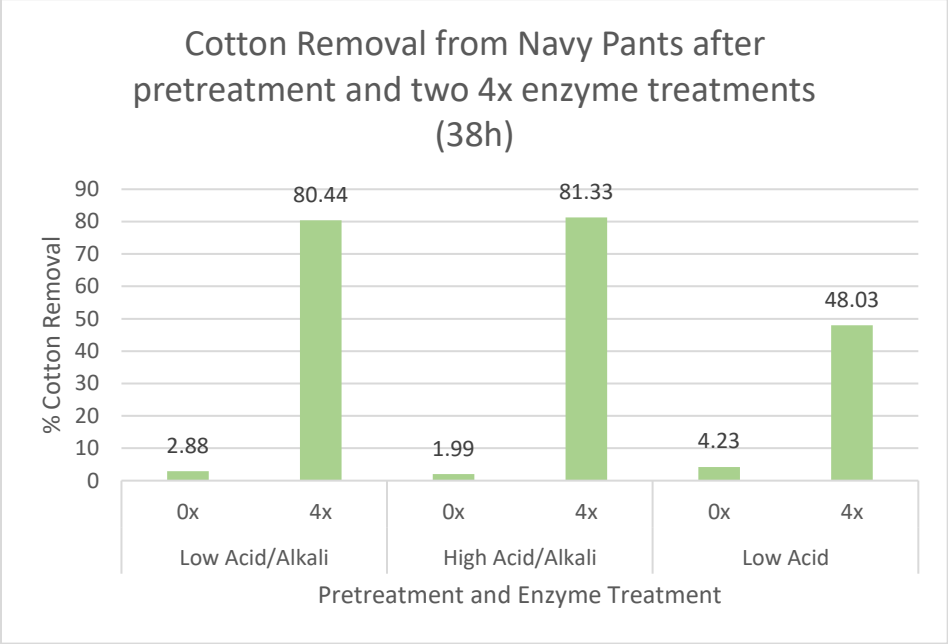


Figure 4.3.6.2.2. Summary of cotton removal from 55/45 modacrylic/cotton navy pants fabric after pretreatment and two 19h enzyme treatments.

4.3.7 Viscose Hydrolysis

A regenerated cellulosic fiber, viscose, was also degraded using cellulase enzymes and was expected to degrade faster than cotton because of its material properties, especially its lower crystallinity which increases the volume of fiber that the enzymes preferentially attack [26]. However, under standard 2mag conditions, the extent of viscose degradation was lower than anticipated (Figure 4.3.7.1). During incubation, the viscose fabric pieces tangled into a fiber "web" where fibers or yarns physically interlinked with each other because of the beating action of the stir bars (Figure 4.3.7.2), which prevented degraded fiber release and reduced enzymatic access to the fibers.

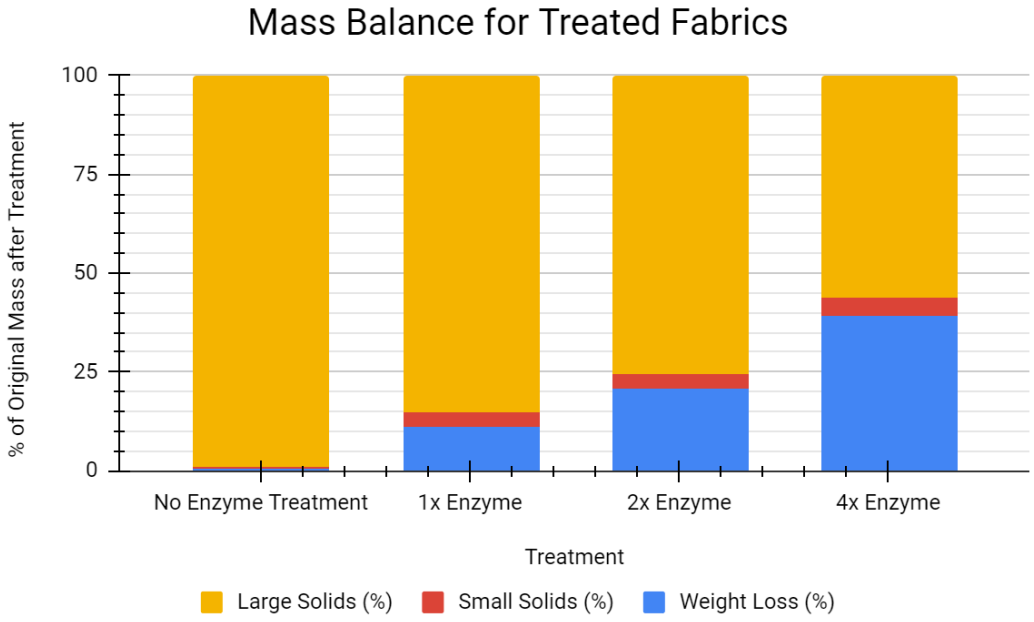


Figure 4.3.7.1. Average degradation of viscose fabric after 19h at standard conditions and varied enzyme doses.

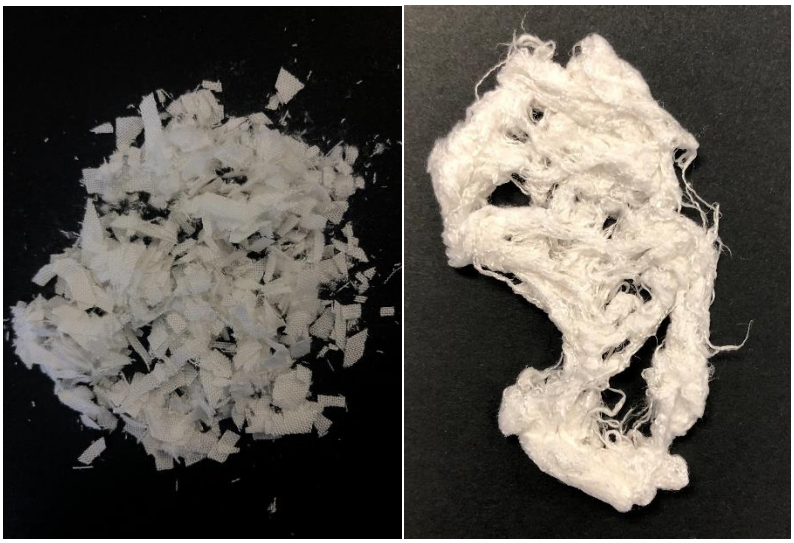


Figure 4.3.7.2. Viscose solids before and after incubation with two stir bars without enzyme addition, showing the reduced swatch size (compared to standard cotton fabric swatch size) used for degradation (left) and the final fiber web that forms because of mechanical agitation (right).

This behavior was very different from the previous cotton fabrics and could be a result of viscose fiber length or otherwise fabric construction. Where most cotton fabrics tested were knits, the viscose was a woven challis, so some yarns would fall out of the sides of the cut swatches before any enzymatic degradation or exposure to magnetic stirring. Even for the control sample shown in Figure 4.7.3.2, the fabric unraveled and then tangled into a web because of the stir bars alone. This same behavior was not observed in the previously tested woven navy pants fabric, which did show some whole yarn release at the edges, but fabric swatches mostly stayed together during incubation. This difference in the two woven fabric behaviors is likely a result of fabric density, where the navy pants fabric was denser, meaning there was more yarn cohesion holding the structure together whereas the viscose fabric was much less dense, so the fabric pieces fell apart under any stirring conditions. Investigation into enzymatic degradation response to both knit and woven fabrics of different densities would be important future work.

Variations from standard 2mag conditions were explored, including adjustment to liquor ratio, fabric swatch size, and mechanical agitation. Ultimately, the most important factor preventing viscose hydrolysis was seen to be mechanical agitation. Even with one stir bar, viscose showed minimal degradation (Figure 4.3.7.3) because of fiber entanglement during mixing. Removing agitation entirely led to more efficient viscose hydrolysis, which was opposite to efficient cotton fabric hydrolysis (see Appendix A-5). The fabric swatch size was also decreased so hydrolysis would proceed more rapidly despite the removal of agitation (Figure 4.3.7.2). Using the smaller fabric pieces without agitation at a 2x dose, the viscose fabric yielded about 75% degradation after 19 hours, with most of the degradation products being soluble components (Table 4.3.7). After 45 hours of treatment, samples treated with a 2x dose were 98% converted from fabric into soluble components, with minimal small solid content collected (Table 4.3.7, Figure 4.3.7.3).

Given the high extent of degradation after 19 hours and the big jump between sample incubation times, it may be possible to achieve this result in less than 45 hours. Since viscose primarily degraded to soluble components (sugars), enzymatically degraded viscose could be highly useful as a fermentation feedstock, but this fiber type would generally have less circular recycling opportunities than cotton fabrics through this process because of the absence of recalcitrant fiber fragments.

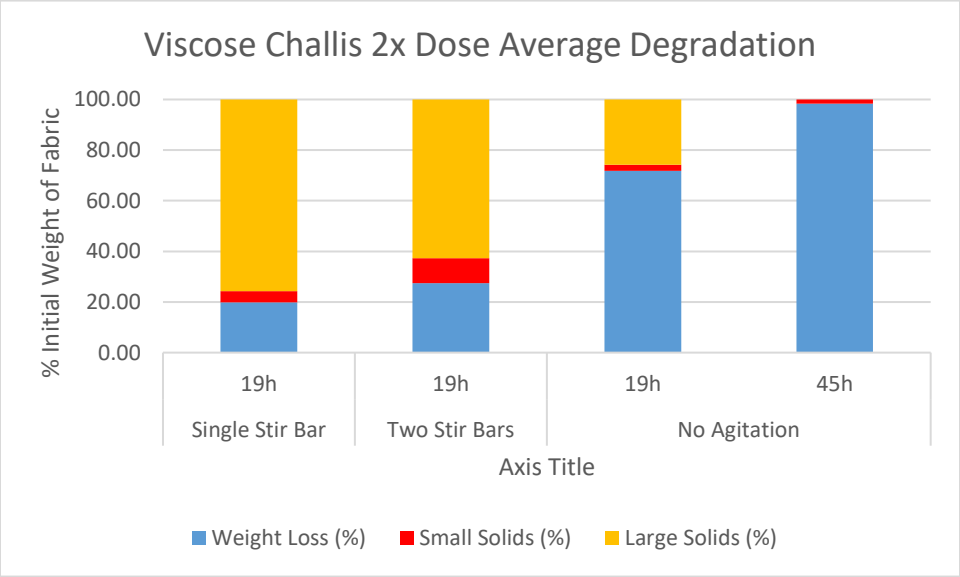


Figure 4.3.7.3. Agitation and time response of viscose challis fabric, all at a 2x enzyme dose.

Table 4.3.7. Average gravimetric data of viscose challis degradation products after specified agitation and incubation length conditions, all at a 2x enzyme dose.

Agitation	Time (h)	Weight Loss (%)	Small Solids (%)	Large Solids (%)
Single Stir Bar	19	19.85	4.41	75.74
Two Stir Bars	19	27.48	9.76	62.75
No Agitation	19	71.81	2.41	25.78
	45	98.41	1.59	0.00

4.4 Weight Loss Characterization

4.4.1 LC-CAD Glucose Analysis

Analysis of clarified liquids recovered from enzymatically degraded bleached cotton samples was carried out through LC-CAD analysis to determine what small molecule degradation products are released, as it was mainly assumed to be glucose before testing. In the LC elution methodology, glucose standard samples consistently showed peaks at retention time of 2.35 minutes. Peaks for enzyme-containing degradation samples showed this same peak, some with added peaks at 1.83 minutes and above 3 minutes which were connected to known pentose and disaccharide elution times. Syrup samples from 1x and 2x dose degradations showed less disaccharide signal than 4x dose samples (Table 4.4.1.1). This reduced presence could be because the samples either did not contain those compounds or otherwise contained them in a quantity below the detectable value. The pentose-corresponding peak was present in all enzyme-containing samples, and it was concluded that it must be a component in the enzyme formulation, as pentose release during enzymatic degradation would not be expected. Despite the assumption that the entire gravimetrically measured weight loss content is glucose, disaccharide peaks in some quantity were expected. If the enzymatic treatment continued long enough, those disaccharides would theoretically all be converted to glucose through beta-glucosidase reaction. In all samples, glucose made up greater than 91% of the entire detected peak area (Table 4.4.1.2), so the assumption that the gravimetrically determined weight loss is all glucose is close to the measured values.

Table 4.4.1.1. Peak areas of glucose and other saccharide peaks measured by DAD during HPLC analysis of clarified liquid samples (where A1-A3 are control, A4-A6 are 1x doses, A7-A8 are 2x doses, and A10-A12 are 4x doses).

	Pentose		Glucose		Disaccharide Peaks							
	Peak 1		Peak 2		Peak 3		Peak 4		Peak 5		Peak 6	
Sample	Time	Area	Time	Area	Time	Area	Time	Area	Time	Area	Time	Area
A1												
A2												
A3												
A4	1.83	0.017	2.34	3.352	3.1	0.038					4.26	0.144
A5	1.83	0.008	2.35	2.887	3.11	0.025					4.25	0.112
A6	1.85	0.006	2.35	3.077	3.11	0.033			3.84	0.004		
A7	1.83	0.027	2.35	4.316	3.1	0.05	3.59	0.025	3.88	0.007	4.28	0.22
A8	1.83	0.021	2.35	4.399	3.1	0.047					4.26	0.205
A10	1.83	0.025	2.36	6.089	3.1	0.094	3.64	0.025	3.85	0.014	4.28	0.349
A11	1.83	0.029	2.34	6.444	3.1	0.095	3.64	0.024	3.87	0.019	4.28	0.393
A12	1.81	0.03	2.35	5.91	3.1	0.095	3.61	0.052	3.86	0.012	4.31	0.356

Table 4.4.1.2. Calculations for percent glucose in matrix using total peak area signals and glucose peak area.

Sample	Glucose Peak Area	Sum of Peaks	Percent Glucose
A1	0	0	N/A
A2	0	0	N/A
A3	0	0	N/A
A4	3.352	3.551	94.40
A5	2.887	3.032	95.22
A6	3.077	3.12	98.62
A7	4.316	4.645	92.92
A8	4.399	4.672	94.16
A10	6.089	6.596	92.31
A11	6.444	7.004	92.00
A12	5.91	6.455	91.56

Glucose concentrations of each sample were calculated using peak areas (Table 4.4.1.3) according to a calibration curve generated by measurement of known glucose standards (Figure

4.4.1.1). These calculated concentration values were compared to predicted values based on gravimetric data for each sample, calculated using Equation 3.5.2.2 (Table 4.4.1.4). Lower enzyme dose and lower glucose concentration samples had much higher percent difference between the two values (Equation 4.4.1) than samples with higher concentration, where the 1x enzyme dose samples showed percent difference between 13-26% (Table 4.4.1.5). More agreement was seen in the 4x dose samples, which had percent difference values of less than 9% (Table 4.4.1.5).

Table 4.4.1.3. Calculation of glucose concentration in degradation samples using peak area and corrections for glucose found in enzyme-only samples.

Dose	Sample	Peak Area	Calculated Concentration (mM)	Enzyme Correction (mM)	Corrected Concentration (mM)
Control	A1	0	0	0	0
	A2	0	0	0	0
	A3	0	0	0	0
1x	A4	3.352	43.26	4.61	38.65
	A5	2.887	35.74	4.61	31.13
	A6	3.077	38.81	4.61	34.2
2x	A7	4.316	58.84	8.98	49.86
	A8	4.399	60.18	8.98	51.2
4x	A10	6.089	87.49	19.19	68.3
	A11	6.444	93.23	19.19	74.04
	A12	5.91	84.60	19.19	65.41

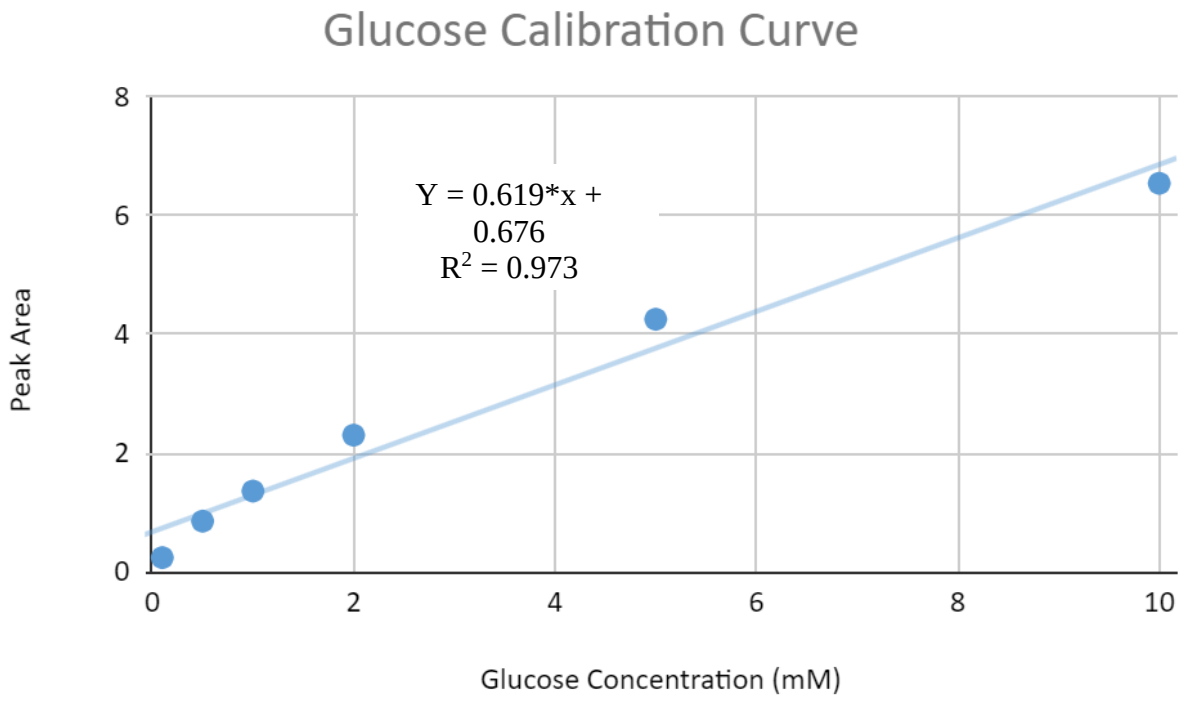


Figure 4.4.1.1. Calibration curve generated correlating peak area measured by HPLC analysis to known glucose standard concentration.

Table 4.4.1.4. Predicted glucose content in g/L and mM for each sample based on calculations with Equation 3.5.2.1 using weight loss data.

Sample	Weight Loss (%)	g/L	mM
A1	0.07	0.04	0.22
A2	0.16	0.09	0.49
A3	0.07	0.04	0.22
A4	9.61	5.34	29.66
A5	8.54	4.74	26.36
A6	9.7	5.39	29.94
A7	14.6	8.11	45.06
A8	15.82	8.79	48.83
A10	24.17	13.43	74.60
A11	25.04	13.91	77.28
A12	21.56	11.98	66.54

$$\% \, Difference = \frac{|predicted - measured|}{\left(\frac{predicted + measured}{2}\right)} \cdot 100$$

Equation 4.4.1 (not calculated for control samples because the value is always 200%.

Table 4.4.1.5. Percent difference (Equation 4.4.1) between gravimetrically predicted glucose concentration and measured values by HPLC.

Sample	Predicted Glu Yield (mM)	Measured Glu Yield (mM)	Percent Difference (%)
A1	0.22	0	N/A
A2	0.49	0	N/A
A3	0.22	0	N/A
A4	29.66	38.65	26.32
A5	26.36	31.13	16.60
A6	29.94	34.2	13.29
A7	45.06	49.86	10.11
A8	48.83	51.2	4.74
A10	74.60	68.3	8.82
A11	77.28	74.04	4.29
A12	66.54	65.41	1.72

A correlation plot between the LC-CAD measured values and gravimetrically predicted values for concentration showed a nearly 1-to-1 relationship, as the trendline had a slope of about 0.96 (Figure 4.4.1.2). This relationship was seen to be strong, with an R^2 value of 0.981. These results further emphasize that for an undyed cotton sample, the gravimetric weight loss value is a good estimator for glucose content in the clarified liquid byproduct. For reactive-dyed samples, this becomes more complicated and generates more byproducts, which will not be discussed further here.

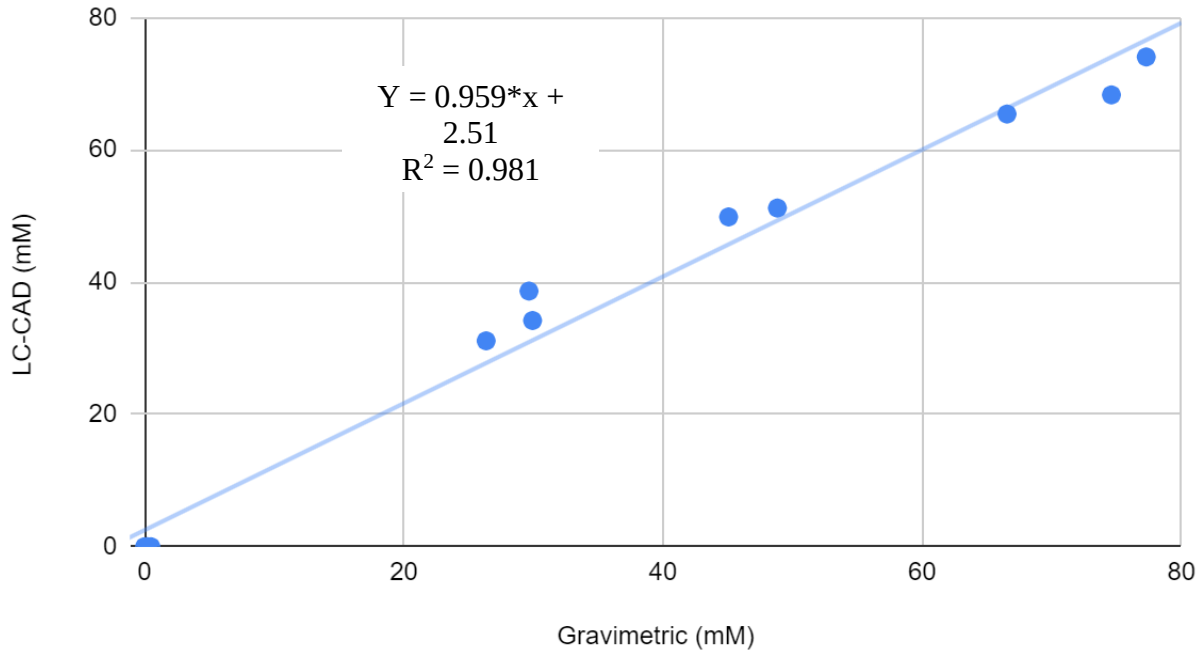


Figure 4.4.1.2. Scatter plot of correlation between predicted glucose concentration based on gravimetric analysis and measured glucose concentration based on LC-CAD analysis

4.5 Small Solids Characterization

4.5.1 Fiber Quality Analysis

Fiber quality analysis [FQA] is a technique that measured the length of 10,000 fibers passing by a detector in a dilute water slurry. This test was conducted on small solids collected as slurries after enzymatic incubation, using all of the samples from the time-response trial (see Section 4.3.5), to understand relationships between fiber length and degradation over time. Results were reported as weighted average length (L_w), which showed a general decrease over longer incubation times for most fabric types, most prominently for fabrics that were resistant to degradation like RC and DP (Figure 4.5.1.1). Notably, the minimum fiber length measured for each sample was 29.5um, which is the minimum detectable length for FQA testing while most samples had maximum measured fiber length greater than 1mm. Because the lower limit was detected for each sample, it was concluded that smaller fiber fragment particle sizes were present

but could not be accounted for in this data. It was useful to generate histograms (truncated at maximum length of 300µm) to illustrate the skewed distribution where a majority of fibers fell under 100µm in length (Figure 4.5.1.1) for all samples, despite 19-hour samples having L_w values of up to 501µm (see values indicated on histograms of fiber lengths for each fabric in Figure 4.5.1.1).

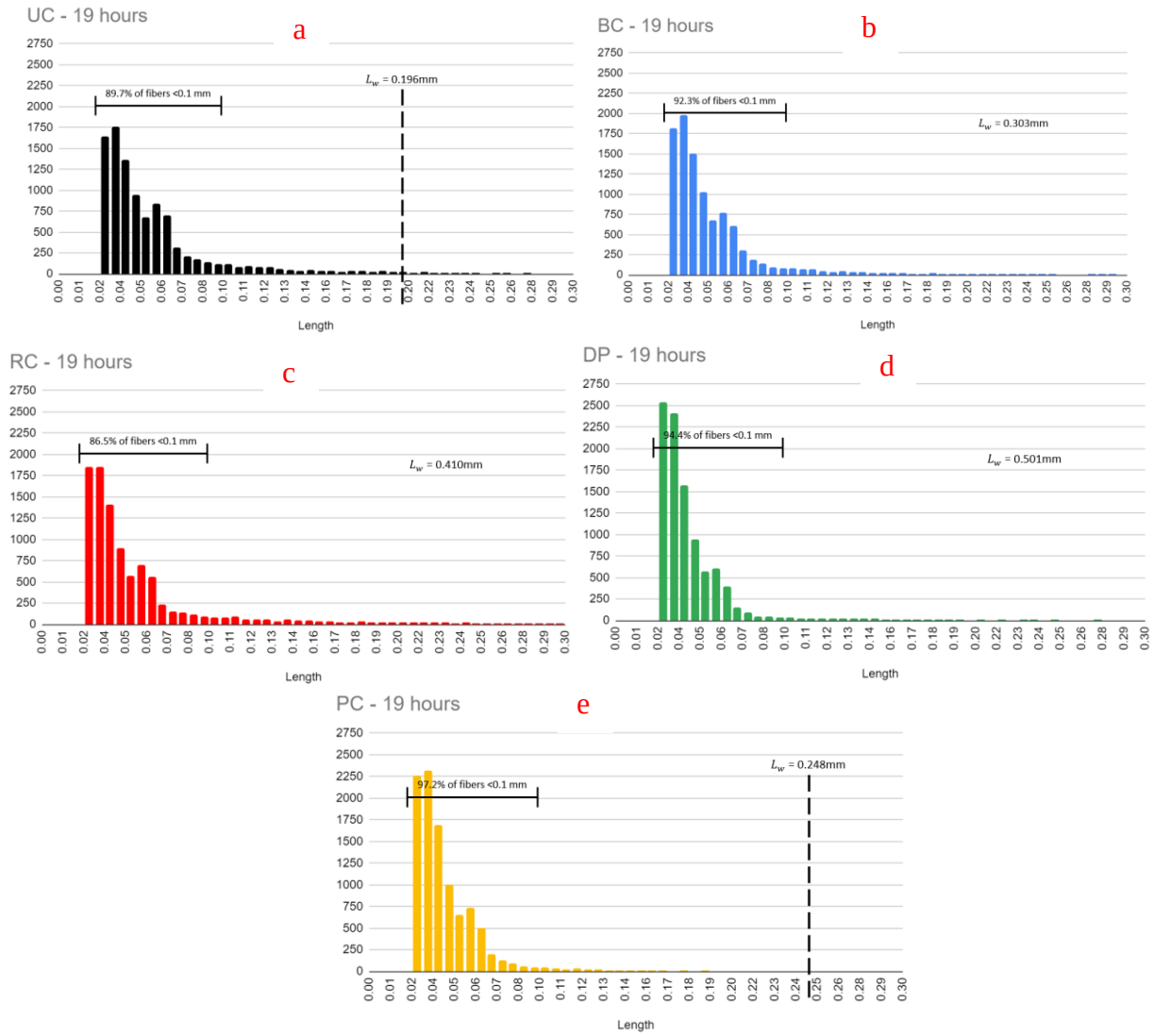


Figure 4.5.1.1. Histograms of length for (a) UC, (b) BC, (c) RC, (d) DP, and (e) PC cotton fibers measured by FQA using recovered small solids after a 4x dose 19-hour treatment. The length weighted average (L_w) and percent of fibers that fall below 100µm are marked on each histogram.

L_w results correlated inversely with gravimetric degradation results (Figure 4.5.1.2), where the sample that was most readily converted to slurry (UC) was the sample with the lowest resulting L_w for its slurry fragments (~200µm), whereas the most difficult to degrade sample (DP fabric) had longer fragments in the degraded slurry (~500µm) (Figure 4.5.1.3). This inverse correlation existed at all treatment times, including for DP and RC samples, which were gravimetrically observed to have different degradation rates after the 45 hour samples (see Figure 4.3.5). Similarly, the FQA data shows that at 19 and 45 hours, when RC showed higher conversion to slurry than DP, it also showed smaller length than DP (Table 4.5.1.1). After 70 hours, when DP conversion to slurry is greater than RC, the DP fibers are shorter (L_w of 266µm versus 300µm for RC). At 96 hours when both samples are fully degraded, FQA shows similar lengths for both fibers, ending around 218µm (Table 4.5.1.1).

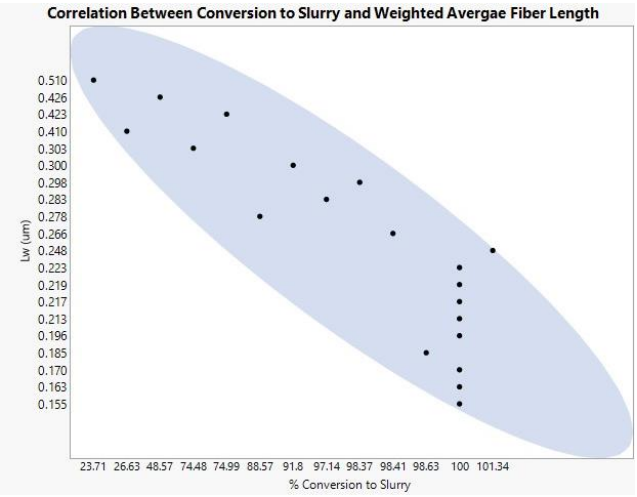


Figure 4.5.1.2. Correlation between fabric conversion to slurry and weighted average length for all samples tested through FQA analysis.

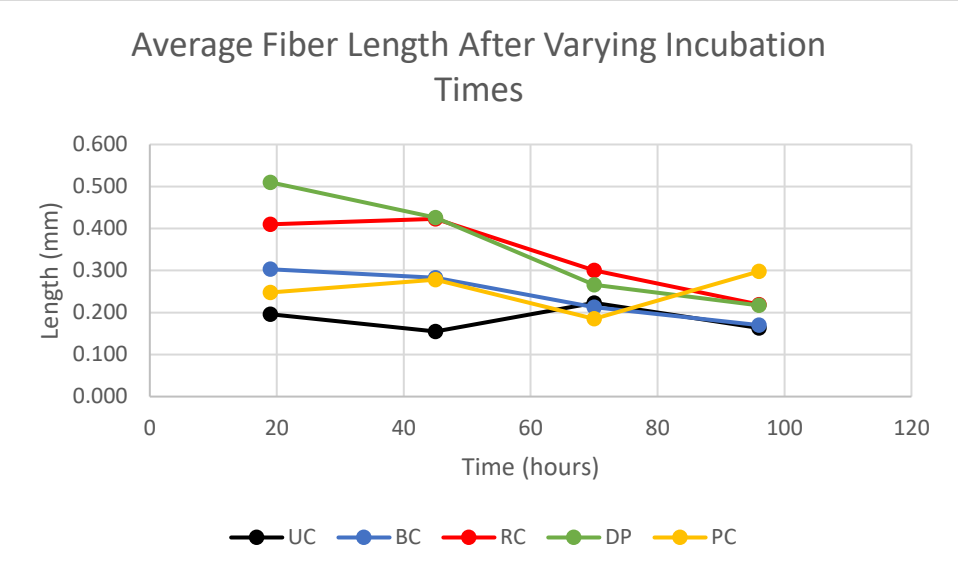


Figure 4.5.1.3. Weighted average fiber length of small solids from various samples after different incubation times.

Table 4.5.1.1. Weighted average fiber length of small cotton solids from different fabrics after a 4x enzyme dose and different incubation lengths, as measured by FQA.

	Length Weighted (mm)				
Time (h)	UC	BC	RC	DP	PC
19	0.196	0.303	0.410	0.510	0.248
45	0.155	0.283	0.423	0.426	0.278
70	0.223	0.213	0.300	0.266	0.185
96	0.163	0.170	0.219	0.217	0.298

All samples trended toward a shorter L_w at longer treatment times, appearing to level off at around 200µm for all 100% cotton samples that were fully converted to a slurry, which includes all cotton samples after 96 hours of treatment (Figure 4.5.1.3). The PC data was much more variable and less patterned than other fabric types. Given the degradation observed at each incubation length, the results for PC45 and PC70 make sense, where PC45 showed less degradation and longer fiber length than PC70 (see Table 4.3.5 above). However, PC19 showed the highest level of cotton removal, and PC96 showed a comparable level of cotton removal to

PC70, and the inverse relationship to fiber length based on this slurry conversion is not observed from the FQA data. Possibly, some polyester shedding occurred in these samples, which gave longer fiber lengths than degraded cotton fragments. For example, PC19 and PC96 had maximum fiber lengths of 1.008mm and 2.01mm, respectively, while PC70 had a maximum fiber length of 0.65mm. It was above concluded (see Section 4.3.5) that PC19 likely had some polyester fiber shedding because of an abnormally high level of mechanical agitation, so it could be that the FQA fibers tested also contained some polyester fibers that increased the L_w. Possibly, the extended 96 hour treatment also showed similar polyester shedding because of the extent of the fabric beating during treatment, leading to an artificially high weighted average.

Fiber length distribution composition over time was examined to observe trends between fabrics and explore different fabric behaviors based on the percentage of fibers that fell below a specified length. 50µm was chosen as that fiber length boundary because it allows for an examination of a majority of the fibers in all cases, while providing enough variation between samples such that conclusions could be drawn. For example, a higher boundary like 100µm includes about 90% of fibers for almost all samples, so very little variation between samples is observed. A 50µm boundary encompassed as little as 57% of fibers for some distributions, so more trends were seen. Also, visual observation of the histograms of fiber length (Figure 4.5.1.1) showed a general trend across all fabric types where most fabrics had a “dip” in fiber frequency around 50um that increased again around 60um. The variable for percentage of fibers that fall below 50um was herein defined as BL50 for “Below Length of 50”. Figure 4.5.1.4 and Table 4.5.1.2 show results for BL50 for all fabric samples. Despite there being no observed correlation between BL50 and fabric conversion to slurry (Figure 4.5.1.5), the relationships between samples from the same fabric over time are useful for exploring fabric behaviors.

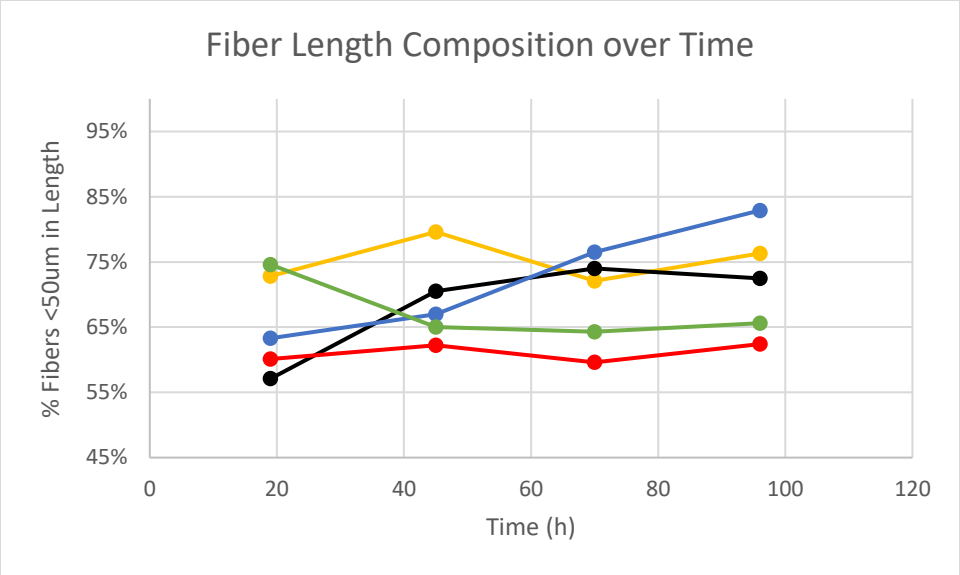


Figure 4.5.1.4. Percent of fibers measured by FQA below 50um for each tested fabric type over time.

Table 4.5.1.2. Percent of fibers from recovered small solids less than 50µm in length measured by FQA for different fabric types after different incubation periods.

	Percent Fibers <50µm in Length				
Time (h)	UC	BC	RC	DP	PC
19	57.1	63.3	60.1	74.6	72.8
45	70.5	67	62.2	65	79.6
70	74	76.5	59.6	64.3	72.1
96	72.5	82.9	62.4	65.6	76.3

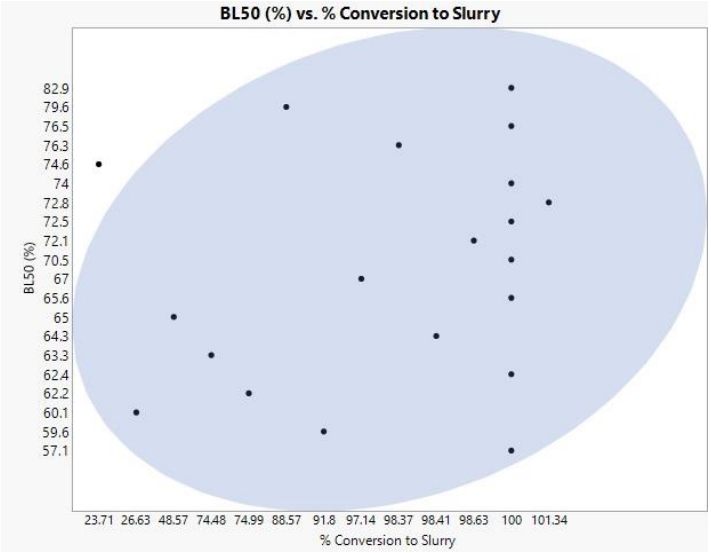


Figure 4.5.1.5. Correlation plot between BL50 values and conversion of fabric to slurry for each sample measured using FQA.

UC sample BL50 data points showed a plateauing effect (Figure 4.5.1.4), where the UC19 sample had 57% and the remaining samples hovered around 72% (Table 4.5.1.2). The 57% BL50 value is the lowest observed for any fabric type, which could be attributed to the way this fabric is able to fall apart more easily than other fabric types. Because the enzyme in combination with the mechanical agitation breaks down UC more rapidly than other fabric types, it could be that the enzymatically damaged structure disentangling itself before the enzyme reduces the fiber length, leaving a more “robust” fiber distribution than is seen in other samples, despite its still extremely skewed distribution.

The observed plateau could be a consequence of a theoretical fiber length limit below which fibers are no longer disintegrated by mechanical agitation, which is a known aid in solids-size reduction during this process. Perhaps below a certain fiber length, the small solids are no longer influenced by the stir bars but are rather only influenced by interaction with the enzyme. Consequently, the fibers would be disintegrated to a theoretically consistent particle size, causing a majority of recovered fine fibers to fall below that size. The FQA data does not indicate any fiber

length as being that theoretical particle size. For the purpose of certain end uses, like isolating cellulose nanocrystals, that fiber length-response to mechanical agitation would be an important factor to understand.

The same plateau effect seen in the UC data was not observed for BC samples, which had BL50 values that increased with every incubation length increase (Figure 4.5.1.4). BC96 has the highest BL50 value at 83% (Table 4.5.1.2). Perhaps this is an artifact of sampling, as 10,000 fibers is a small portion (around 0.25mL) of one collected slurry, which is about 12mL of suspended solids. It is possible that the fibers tested for BC96 were a non-representative sample, or perhaps there is some other effect that is not clear at this time. BL50s for RC were very stagnant, with about 60% falling below 50µm at every incubation time (Figure 4.5.1.4). This consistency could indicate a general lower limit that the crosslinked fiber segments are able to degrade to in general, where any stubborn small solids that do not convert into soluble components may have crosslinks that prevent them from degrading to particle sizes below the "lower limit". Outside of UC19, all four RC samples have the lowest BL50 values (Table 4.5.1.2).

DP fabrics showed an inverse plateau effect for BL50 values (Figure 4.5.1.4), where the DP19 sample showed 75% and the DP samples from the other three incubation lengths showed 65% (Table 4.5.1.2). It was assumed that the acid pretreatment caused some fiber damage on top of the observed fiber shedding, which could mean that a significant amount of pre-damaged short fiber content was released from the fabric structure quickly during enzyme treatment, resulting in a higher BL50 for the 19-hour samples. After extended degradation lengths, the immediate release of short fibers was possibly "balanced out" by the longer more stubborn fibers, like the ones observed in RC slurries. For that reason, it is possible that the stagnation of DP at BL50 of 65% being slightly higher than the stagnation of RC at 60% is due to the percentage of pretreatment-

damaged fibers that appear to fall primarily in the BL50 range that are not present in the RC samples. It could be that undamaged, enzymatically degraded DP fibers would also be about 60% BL50.

PC fibers generally showed high BL50 percentages (Figure 4.5.1.4), with all values above 72% (Table 4.5.1.2). The mechanism of fiber release from the knit synthetic/cotton blend structure has been previously described as degradation of cotton fibers to fragments that are small enough to fall out of the knit structure (see Section 4.3.4). Notably, not all released solids are below an "ideal" particle size as some release from fabric edges where there is less restriction in movement and enzyme access. The BL50 data supports that mechanism claim because each sample had a high percentage of short fibers, with generally higher BL50 values than UC fiber samples. Perhaps the particle size that releases most efficiently from the studied 50/50 polyester/cotton blend structure is around 50µm. Knowing the particle size requirement could inform future decisions like end use of small solids or ideal swatch size for rapid release while retaining easily recoverable synthetic solids.

4.5.2 X-ray Diffraction Analysis

X-ray diffraction [XRD] was carried out on enzymatically degraded fine fibers because it gives insight into their crystallinity, which was expected to increase relative to the original fiber crystallinity. XRD plots show peaks in areas where ordered structures are identified by the incident x-rays that strike a sample from different angles over the course of the test. Peak height between 22-23º for cellulose samples can give a rough qualitative indication of sample crystallinity when samples analyzed by the same method are compared such that the higher the peak intensity, the higher the crystallinity of that material in general. For quantitative comparison, there are several ways to calculate crystallinity index (CI) based on XRD data. The typical method is based on a

fundamental study from 1959 by Segal et al., which uses the maximum peak height from the 2Θ range identified as representing crystal response (22-23º) and the minimum intensity from the range understood to represent amorphous response (18-19º) [68].

In research conducted in parallel to the current work, XRD comparisons were made between mechanically milled cotton fibers and enzymatically degraded cotton fiber fragments [69]. It was determined that enzymatically treated UC and BC fibers had higher crystallinity (CI of 90 and 88, respectively) than milled UC and BC fibers (Figure 4.5.2.1), which both had a CI around 73. This indicated that amorphous regions of cotton fibers were enzymatically attacked first, leaving behind more crystalline fragments than could be obtained through mechanical means. A more crystalline product could have higher potential value for varied end uses than a lower crystalline product.

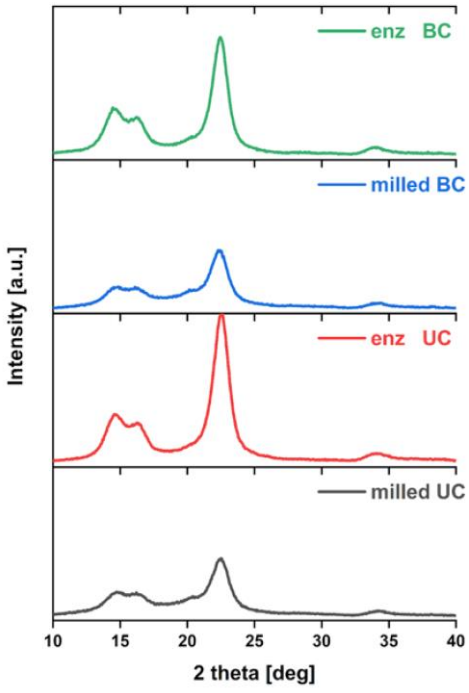


Figure 4.5.2.1. Prior X-ray diffraction results from parallel work that compares cotton fibers collected after enzymatic degradation versus cotton fibers generated by mechanical milling for UC and BC. Reprinted from Wang et al. [69].

XRD analysis was conducted for the current project on small solids recovered after a standard 2mag 19-hour enzymatic treatment using a 4x dose (Figure 4.5.2.2) to investigate the crystallinity of fiber from the different fabric samples examined. Qualitative results showed that crystalline peak intensity decreased in the order of UC > BC > RC > PC > acid-pretreated DP. Crystallinity index calculations for each of these curves are summarized in Table 4.5.2. Crystallinity index decreased in order of UC > BC > PC > RC > DP, which mostly confirms the qualitative observation based on peak intensity and indicates a significant CI increase for all fiber types relative to the milled fabrics above (where all enzymatically degraded fiber types have improved from an assumed CI of 73 to at least 84, Table 4.5.2). The discrepancy between PC and RC placement in the qualitative and quantitative rankings is explained by the relative amount of fiber used for the analysis. The PC sample had less fiber available for analysis than the samples originating from 100% cotton fabrics, so its observed intensity was decreased relative to its mass.

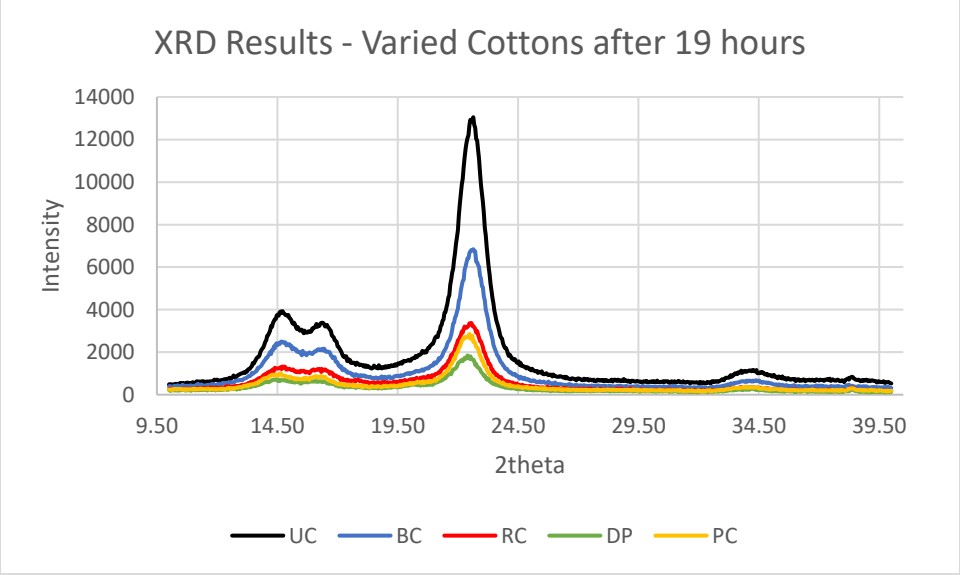


Figure 4.5.2.2. XRD data showing a comparison between small solids (cotton fiber fragments) recovered from 4x dose 19 hour treatment of 100% cotton fabrics: UC, BC, RC, acid-pretreated DP, and PC.

Table 4.5.2. Relevant peak intensities used to calculate the reported crystallinity index. (* - from Wang et al. 2023 [1])

	No Enz*	UC	BC	RC	DP	PC
I_c		13050	6843	3377	1846	2856
I_a		1229	714	519	290	334
Crystallinity Index	74	90.58	89.57	84.63	84.29	88.31

The CI calculated for PC fibers (88.31) was lower than the UC fiber results (90.58), which was not anticipated. Because PC samples are only 50% cellulose, their relative enzyme-to-cellulose ratio is higher than in a UC degradation. Further, the FQA results showed that PC fibers had more fibers of short length after 19 hours of degradation than UC fibers (see BL50 data, Figure 4.5.1.3). Because of these two known characteristics of PC degradation, it was hypothesized that recovered PC fibers would have higher crystallinity than the UC fibers, which theoretically received less concentrated enzymatic attack than the PC. The physical block of the polyester yarns that slowed fiber release from the fabric structure must have also somewhat restricted the preferential amorphous region attack on the released cotton fibers, ultimately yielding a fiber sample with lower crystallinity.

The XRD crystallinity index rankings for 100% cotton fabrics correlate well with gravimetric degradation results for each of the samples (see Section 4.3.5), where UC had the most degradation, followed by BC, RC, and then the acid-pretreated DP samples. The most intense peaks being observed for UC and BC make sense with the previous work, showing that enzyme treatment preferentially degraded the amorphous regions, leaving behind small solids with a high degree of crystallinity. The lower crystallinity of RC and acid-pretreated DP small solids samples implies that enzymes were blocked from reaching amorphous regions by the chemical obstacles. Since conversion of BC, RC and acid-pretreated DP to slurry increased with time (according to

Figure 4.3.5), future work using XRD analysis could investigate whether longer treatment times of these samples would lead to higher crystallinity in recovered small solids or whether the obstacles presented by chemical modifications cause a different crystallinity result after more extensive enzymatic degradation. This future work is especially relevant for chemically pretreated DP fibers, which may have incurred fiber damage during the acid treatment step.

The observed consistency between gravimetric data and crystallinity supports the idea that crystallinity of resultant fiber fragments is dependent on the extent of fiber degradation after release from the fabric and not just enzymatic fabric degradation alone. Because degraded fibers from a sample that is resistant to degradation, like RC or DP small solids, show lesser crystallinity than bleached cotton after the same length of treatment, degraded fiber crystallinity must increase further after release from the large fabric structure. This conclusion is also supported by the CI for the PC fibers, where despite the gravimetric indication that cotton was fully removed from the fabric structure (Table 4.3.5), the CI was lower than UC fibers, indicating that the slowed release of fibers from the fabric structure played a noticeable role in the final fiber crystallinity. This behavior is important to understand when considering potential small solids recycled end use. For example, a high level of crystallinity may be required for using cotton fiber fragments as a strengthening agent in composites. Therefore, some processing decisions for energy or chemical input may ultimately be controlled by intended end use rather than efficient degradation alone.

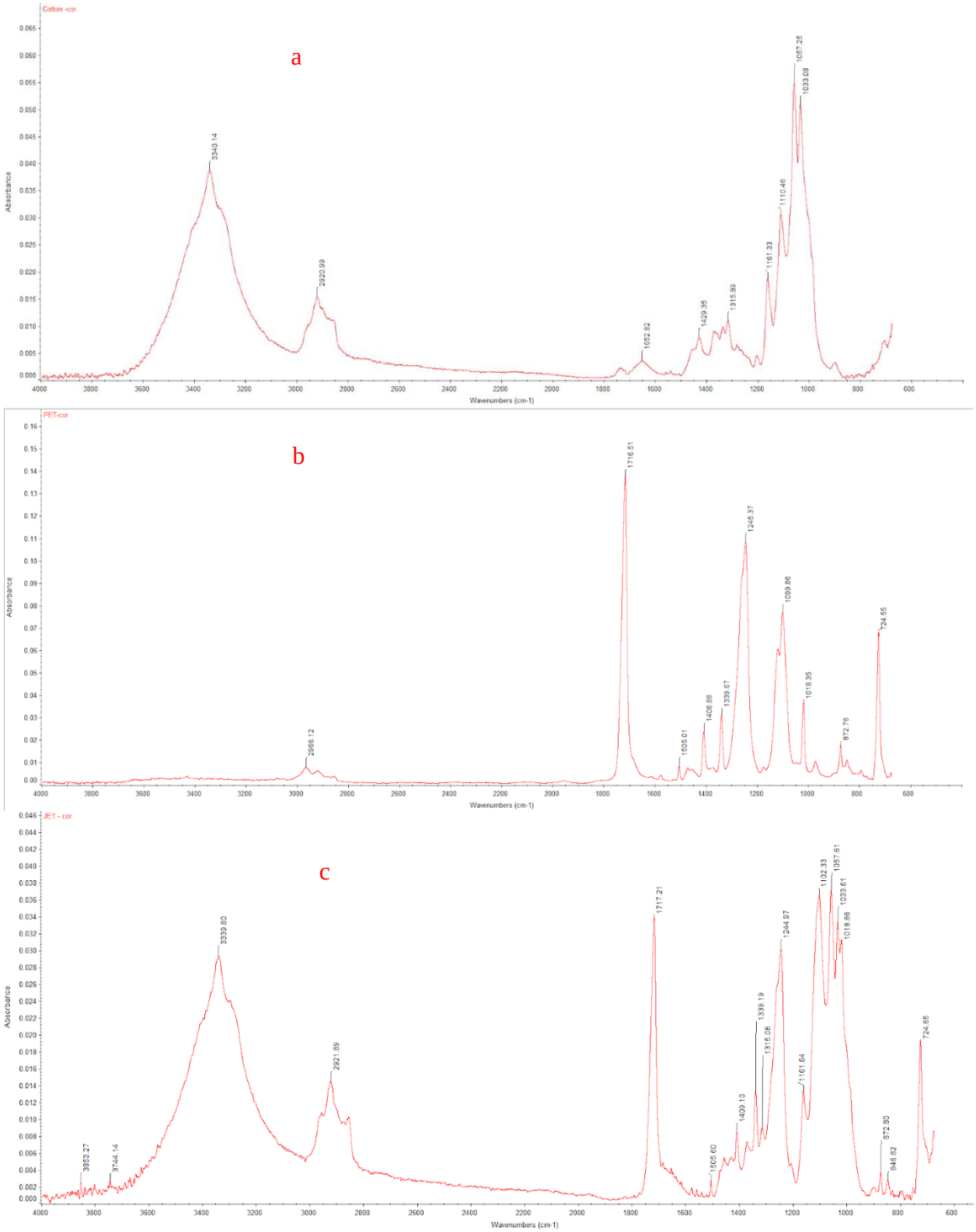
4.6 Large Solids Characterization

4.6.1 Fourier-transform Infrared Analysis

FTIR analysis was carried out to verify the removal of cotton from a fabric sample through enzyme treatment. The resultant spectra for 100% cotton (UC), 100% polyester, 50/50 polyester/cotton (PC), and residual large solids after enzyme treatment of PC at two different doses

are shown in Figure 4.6.1.1. FTIR showed a characteristic broad peak for cotton fabric around 3300 cm^{-1} attributed to -OH stretching, with a smaller peak around 2900 cm^{-1} which is typically attributed to aliphatic and aromatic C-H bond stretching (Figure 4.6.1.1a) [43]. A PET reference sample shared this small peak at 2900 cm^{-1}, but the spectra is mainly composed of sharp peaks in the region below 2000 cm^{-1}, with the most prominent peak occurring at 1720 cm^{-1}, attributed to the ester carbonyl bond [43] (Figure 4.6.1.1b). Untreated PC fabric (Figure 4.6.1.1c) showed a combination of the two individual reference spectra, with a broad peak at 3300 cm^{-1}, sharp PET peak at 1720 cm^{-1}, and an additive combination of peaks below 1500 cm^{-1}.

Figure 4.6.1.1. FTIR spectra of solid fabric pieces from (a) UC, (b) a PET reference, (c) a control treated PC fabric, (d) a PC fabric with gravimetrically measured 80% cotton removal, and (e) a 4x double treated PC fabric with theoretical full cotton removal (gravimetrically, ~94%).


Cotton -cor
a
Absorbance
Wavenumbers (cm-1)
3340.14
2920.99
1652.82
1429.35
1315.89
1161.33
1110.46
1057.25
1033.09
PET-cor
b
Absorbance
Wavenumbers (cm-1)
1716.51
2966.12
1605.01
1406.88
1339.67
1246.37
1099.86
1018.35
872.78
724.55
JE1 - cor
c
Absorbance
Wavenumbers (cm-1)
3939.80
3953.27
3744.14
2921.89
1717.21
1605.60
1439.10
1339.19
1316.06
1244.97
1161.64
1102.33
1057.61
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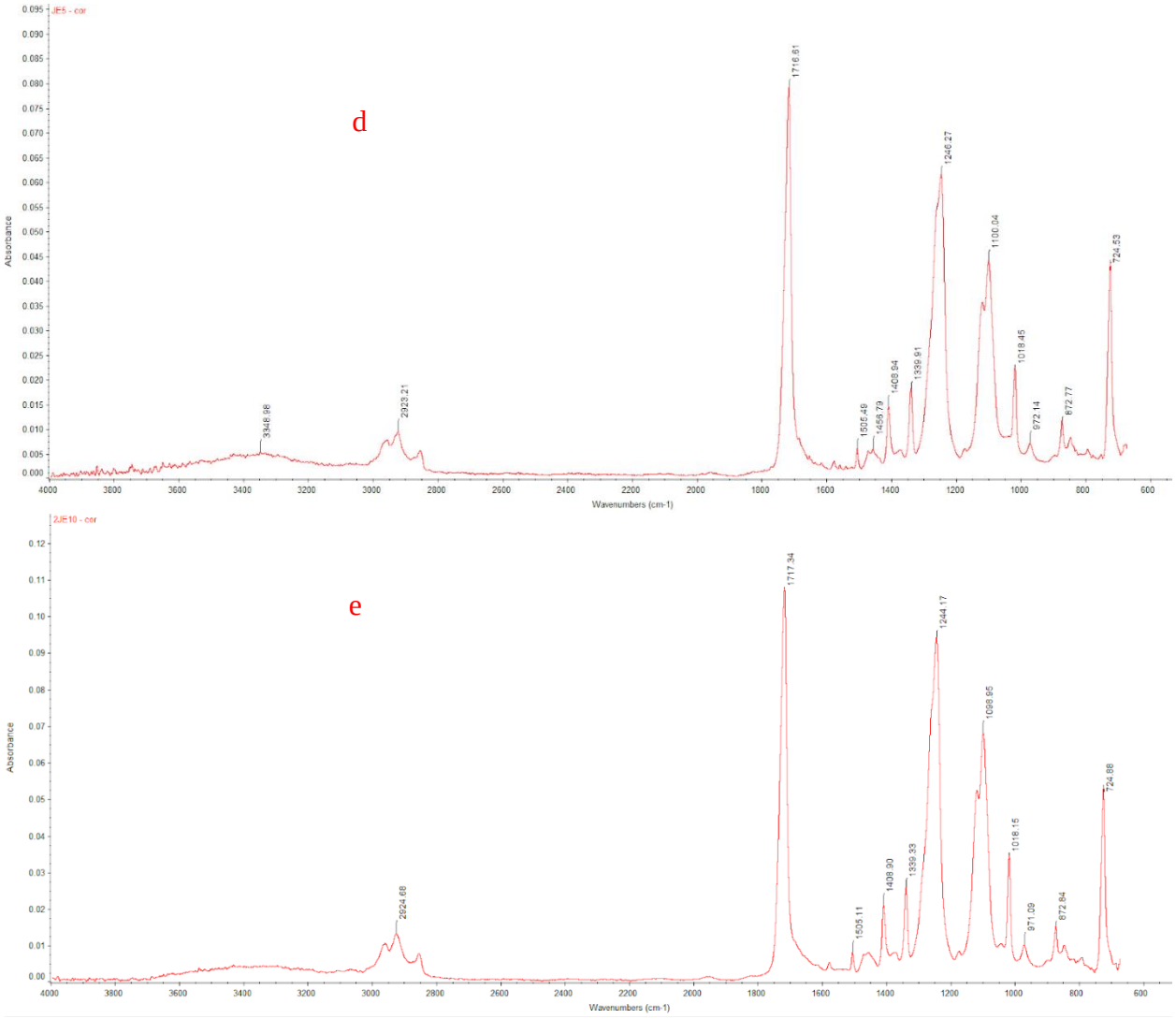
1018.96
872.80
849.82
724.65



FTIR from a residual large solid sample collected after a 1x dose double treatment, gravimetrically measured as 80% cotton removal showed a significant decrease in the broad 3300 peak, though it was still identified by as a peak by the software (Figure 4.6.1.1d). The presence of minimal signal compounding in the fingerprint region was also observed for this sample. The residual 20% of cotton in the fabric was observed, but the signal is not very different from a "cleaned" PET sample. Fabric recovered after a 4x dose double enzyme treatment from the same PC fabric showed the absence of a detected broad cotton peak, though some absorbance around 3300cm^{-1} is seen (Figure 4.6.1.1e). It further contained sharp peaks aligning with those observed

in the PET reference sample, demonstrating that cotton has been substantially removed from the remaining polyester fibers.

Through parallel SEM analysis, it was observed that the cotton remaining in the fabric is typically buried within the highly twisted polyester yarns (right of Figure 4.6.1.2). Because the FTIR-ATR method primarily analyzes the surface of samples, fibers that were buried deeper inside the twisted yarn structure for both the 80% cotton removal and enzymatically cleaned PET samples could have been consequently unaccounted for by the resultant spectra. In the case of the 80% cotton removed fabric, it could also be that the crystal contacted a portion of the sample where the cotton was most significantly removed. It has been previously observed that cotton removal typically occurs from the edges first and occurs slowest in areas where the fabric has folded or curled (Figure 4.6.1.3). Possibly, the sample contacted the crystal near the edge or in an area of the fabric where more cotton was removed. Overall, the enzymatically cleaned polyester sample is almost entirely like the PET reference sample, which could indicate that enough cotton has been removed to prep the recovered synthetic solids for recycling.

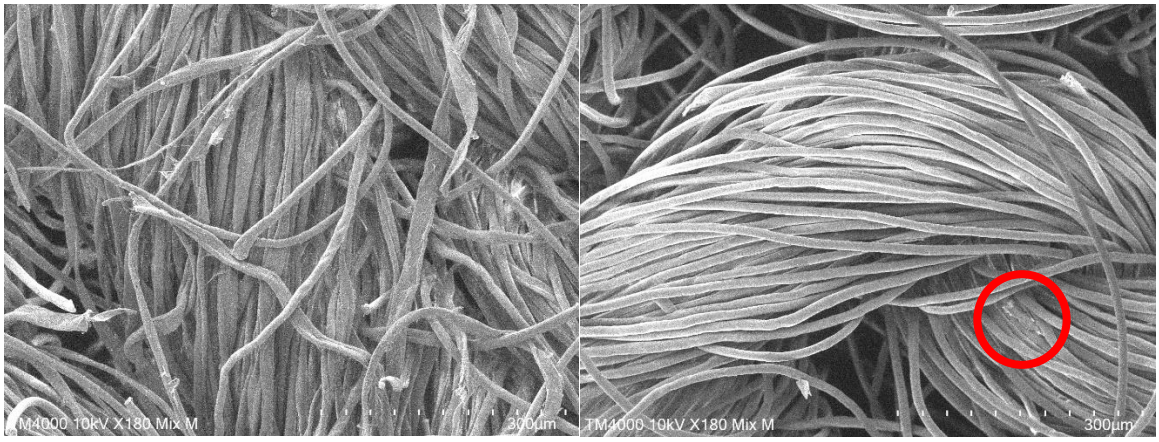


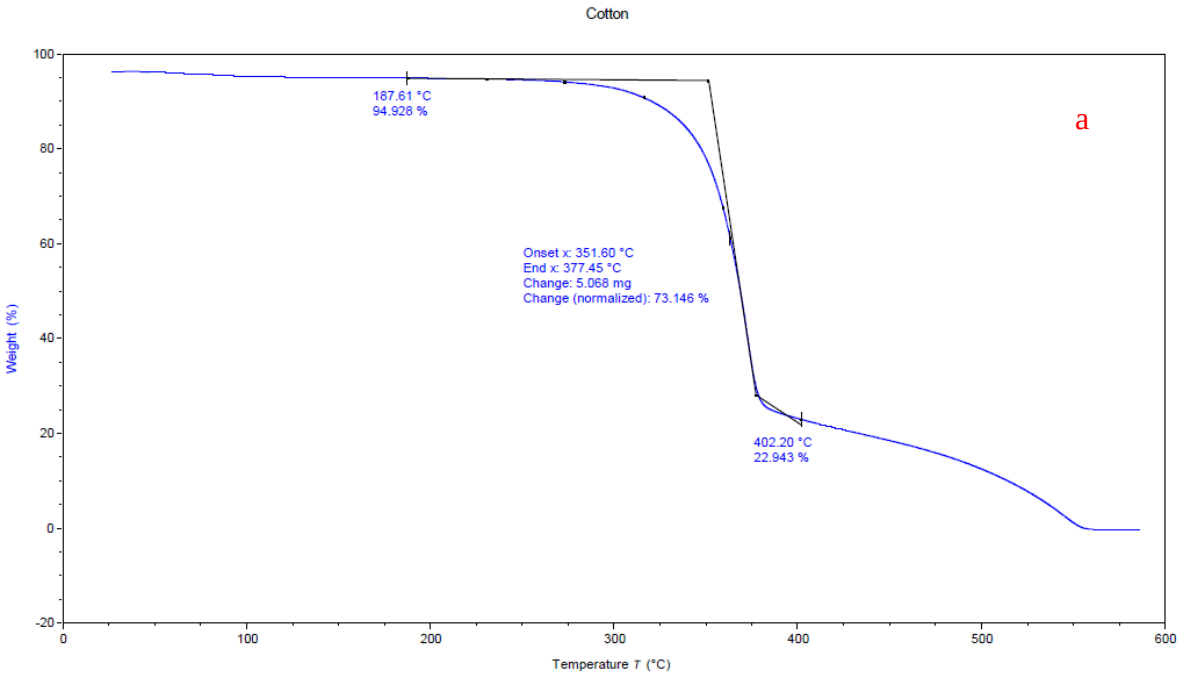
Figure 4.6.1.2 SEM images of untreated PC fabric and enzymatically cleaned PET (gravimetrically, 94% cotton removal, right) where the red circle indicates a buried cotton fiber within the remaining yarn structure (photos by Vince Varju).

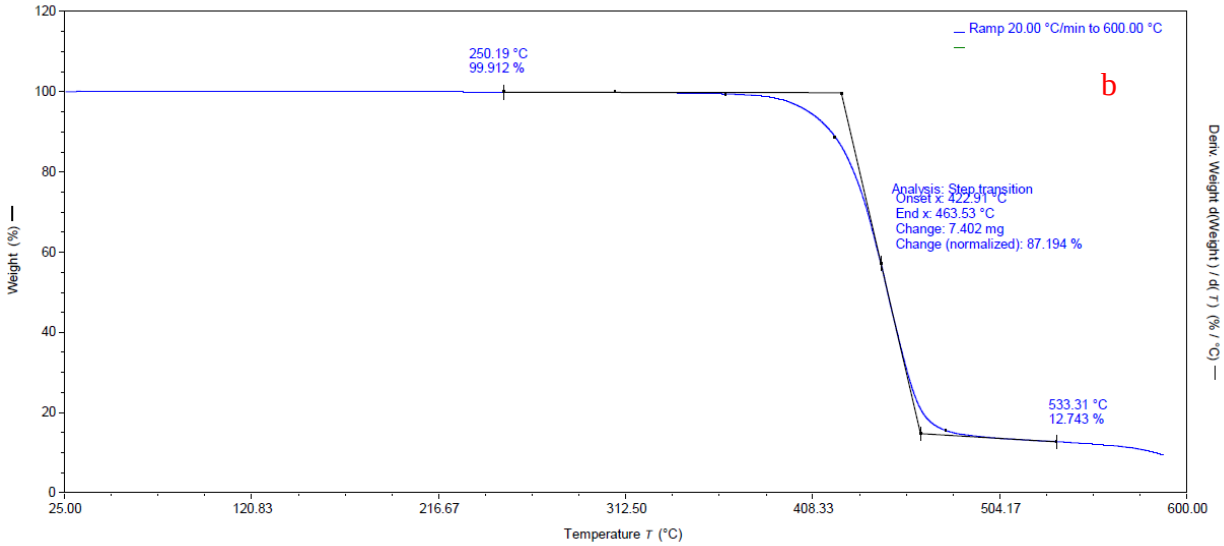
Figure 4.6.1.3 Image of a 50/50 white polyester/blue dyed cotton fabric with partial cotton removal, visually displaying cotton fiber loss from the edges to the middle. Not used for analytical techniques.

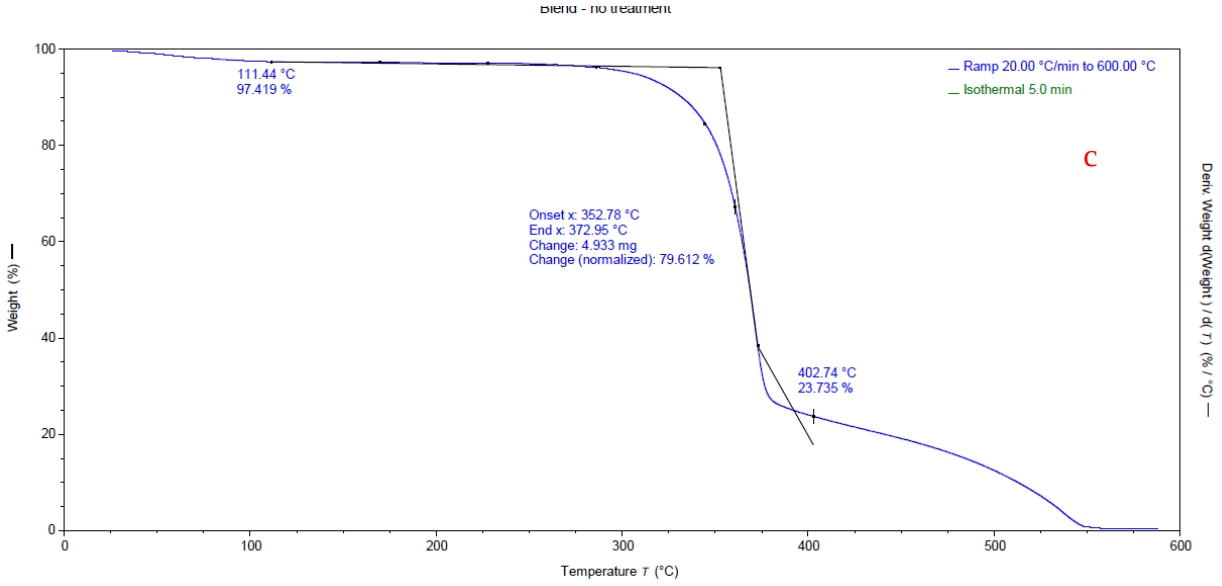
4.6.2 Thermogravimetric Analysis

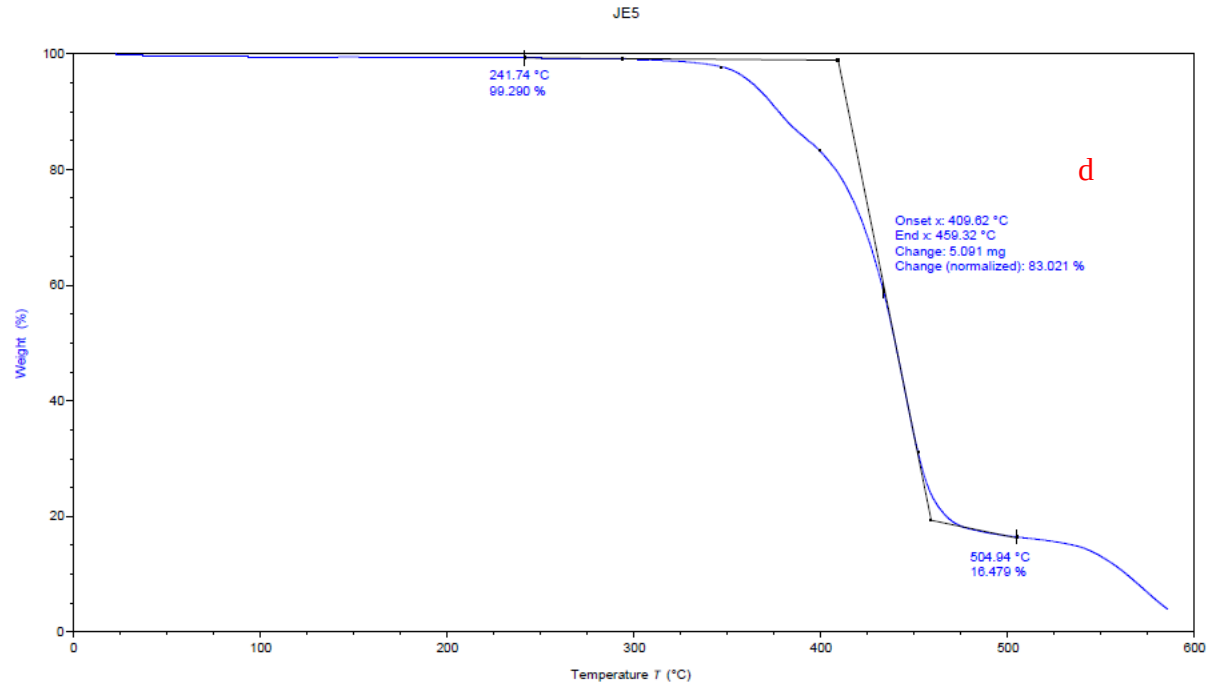
Thermogravimetric analysis (TGA) was carried out to understand the effect of enzyme treatment on polyester degradation temperature in relation to a reference sample. A change in onset temperature after treatment could indicate a reduction in quality. UC fabric analyzed through thermogravimetric analysis (TGA) showed typical cotton behavior [70] with a minor weight decrease around 100°C (Figure 4.6.2a), due to evaporation of water, followed by a step transition at onset temperature of 351.6°C, with about 73% of fabric weight loss in this transition, followed by a slower rate of degradation of the remaining 23% after 377°C. A PET reference also showed typical behavior [44] with onset degradation at 422°C and about 12% residual after degradation (Figure 4.6.2b). Unlike other blended samples, which commonly show two separate step transitions at the degradation temperature of each component, 50/50 PET/cotton showed one step transition with very similar onset and offset degradation temperatures as well as slowed degradation rate after the offset to UC fabric. This was unexpected, as other studies have seen a degradation step transition with noticeable shouldering for polyester/cotton blended samples [71].

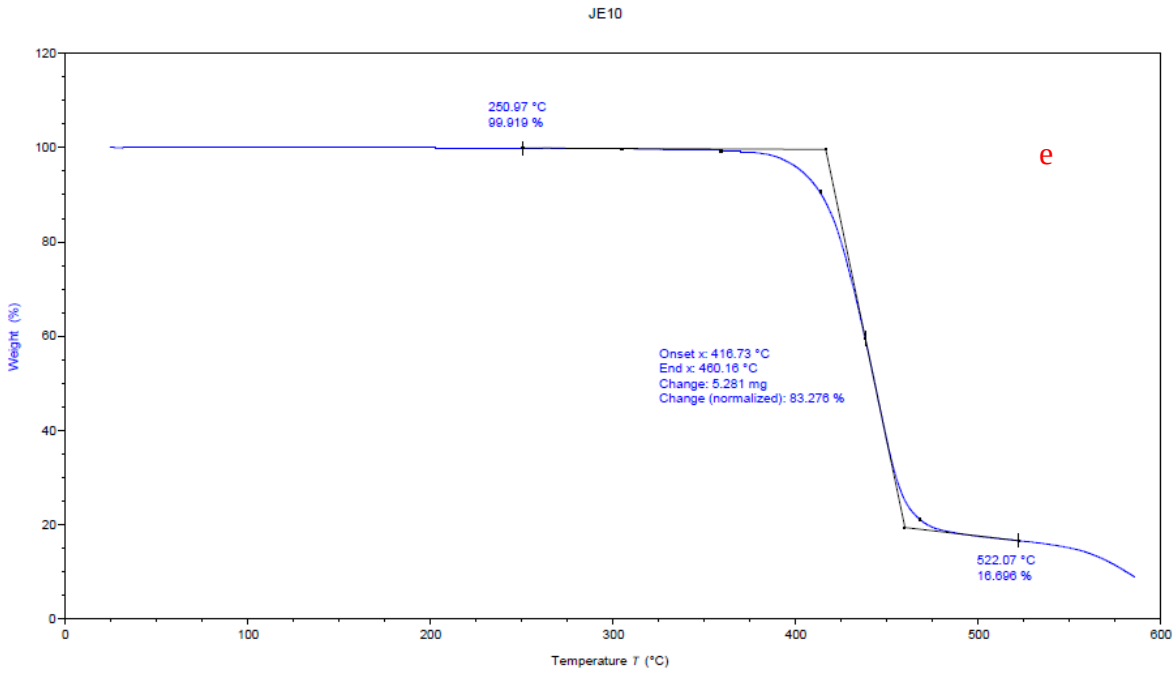
Figure 4.6.2. TGA results from (a) UC, (b) a PET reference, (c) PC control sample, (d) PC 80% cotton removal, and (e) PC full theoretical cotton removal (gravimetric: 94%).


Cotton
187.61 °C
94.928 %
Onset x: 351.60 °C
End x: 377.45 °C
Change: 5.068 mg
Change (normalized): 73.146 %
402.20 °C
22.943 %
a
Weight (%)
Temperature T (°C)


Ramp 20.00 °C/min to 600.00 °C
250.19 °C
99.912 %
b
Analysis: Step transition
Onset x: 422.91 °C
End x: 463.53 °C
Change: 7.402 mg
Change (normalized): 87.194 %
533.31 °C
12.743 %
Weight (%)
Deriv.Weight d(Weight) / d(T) (% / °C)
Temperature T (°C)


Blend - no treatment
Ramp 20.00 °C/min to 600.00 °C
Isothermal 5.0 min
111.44 °C
97.419 %
Onset x: 352.78 °C
End x: 372.95 °C
Change: 4.933 mg
Change (normalized): 79.612 %
402.74 °C
23.735 %
c
Weight (%)
Deriv. Weight d(Weight) / d(T) (% / °C)
Temperature T (°C)


JE5
241.74 °C
99.290 %
Onset x: 409.62 °C
End x: 459.32 °C
Change: 5.091 mg
Change (normalized): 83.021 %
504.94 °C
16.479 %
d
Weight (%)
Temperature T (°C)



A recovered large solid from a gravimetrically measured 80% cotton removal sample showed a more typical polyester/cotton blend degradation curve, with onset temperature around 350°C, and a shoulder after a small weight percentage around 400°C, and an offset degradation around 460°C (Figure 4.6.2d). The shouldered transition indicates cotton presence in the sample, which supports the known gravimetric data that suggests 20% of the original cotton remains in the fabric. TGA of an enzymatically cleaned polyester sample shows thermal behavior that corresponds with the PET reference, with onset degradation at 417°C and about 16.7% solids remaining after the transition (Figure 4.6.2e). No shouldering in the step transition was observed, suggesting the thermal properties of this "cleaned" sample (which is understood to have trace amounts of cotton remaining) seem comparable to the reference sample, which is a good sign for mechanical recyclability of the recovered solids.

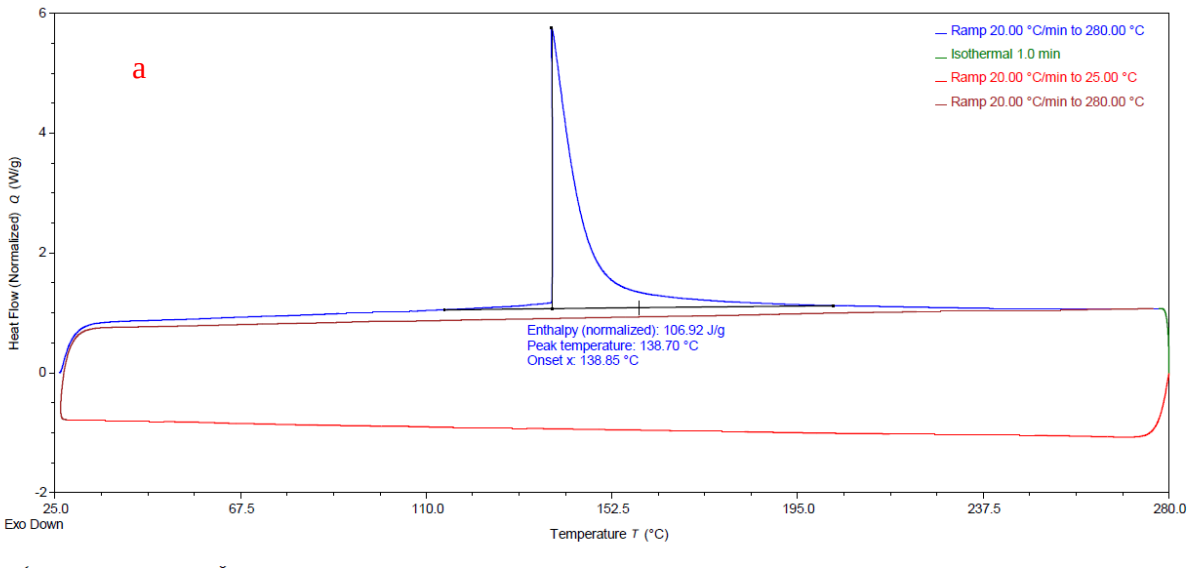
4.6.3 Differential Scanning Calorimetry [DSC] Analysis

DSC analysis was carried out to assess the thermal response of fabrics to controlled heating and cooling cycles, primarily to investigate the response of the recovered enzymatically cleaned

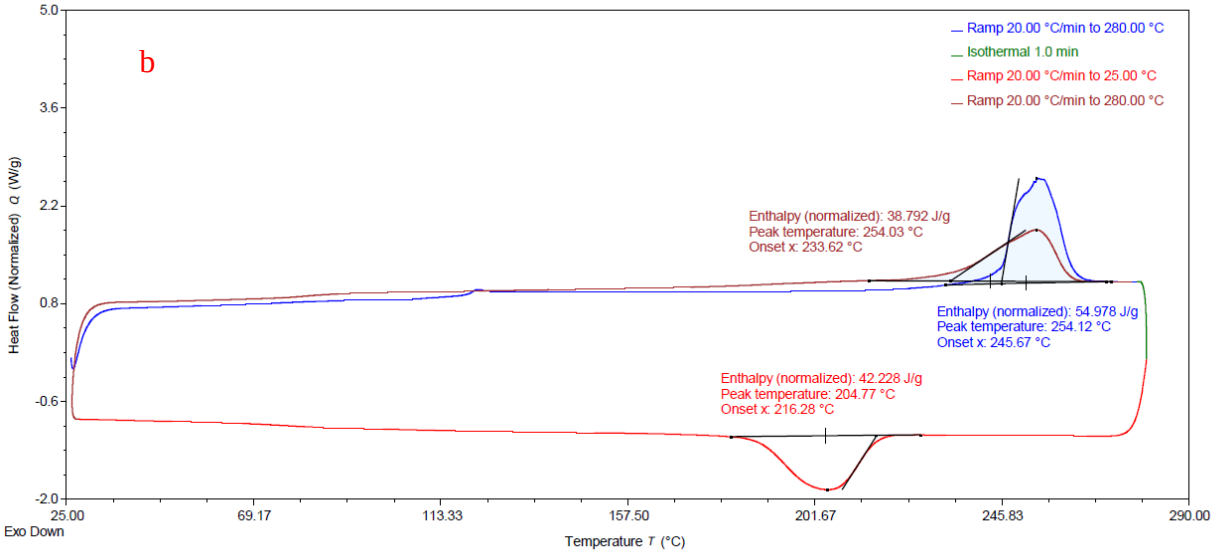
polyester in relation to reference response and blend response. UC fabric was used as a cotton reference and showed a sharp endothermic peak during the first heating cycle at around 135°C (Figure 4.6.3a), which is attributed to water loss [72]. Fibers from a 100% polyester reference fabric had a melting peak at 254°C during both heating cycles, where the first heating cycle (blue) showed a sharper peak with a shoulder and the second heating cycle (burgundy) showed a broader peak with no shoulder (Figure 4.6.3b). These reference peaks were compared to large solids collected from PC samples that were treated without enzyme (Figure 4.6.3c), with a 1x enzyme double treatment (Figure 4.6.3d), and a 4x enzyme dose double treatment (Figure 4.6.3e). The sample without enzyme showed negligible degradation, the sample with 1x enzyme was gravimetrically shown to have 80% cotton removal, and the sample with 4x enzyme was gravimetrically measured to have achieved 94% theoretical cotton removal, with 53% large solids remaining. This sample was commonly referred to as having achieved full cotton removal. A summary of the DSC peak comparisons is shown in Table 4.6.3.

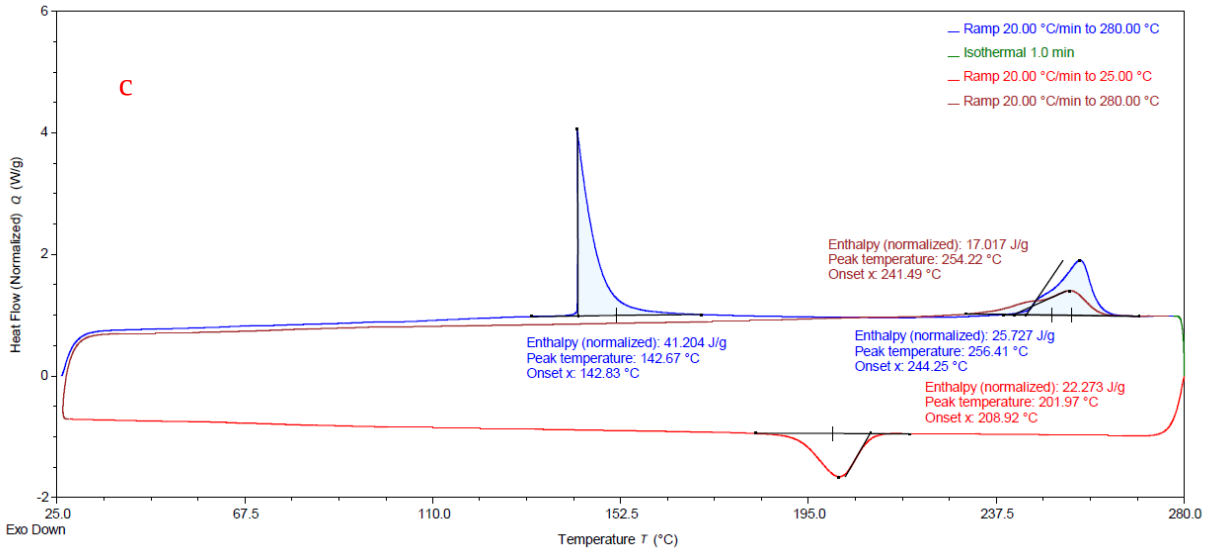
Figure 4.6.3. DSC results from (a) UC, (b) a PET reference, (c) PC control treated sample, (d) PC with partial cotton removal (80%, gravimetric measurement), and (e) PC with theoretically full cotton removal (94%, gravimetric measurement).

Cotton - untreated

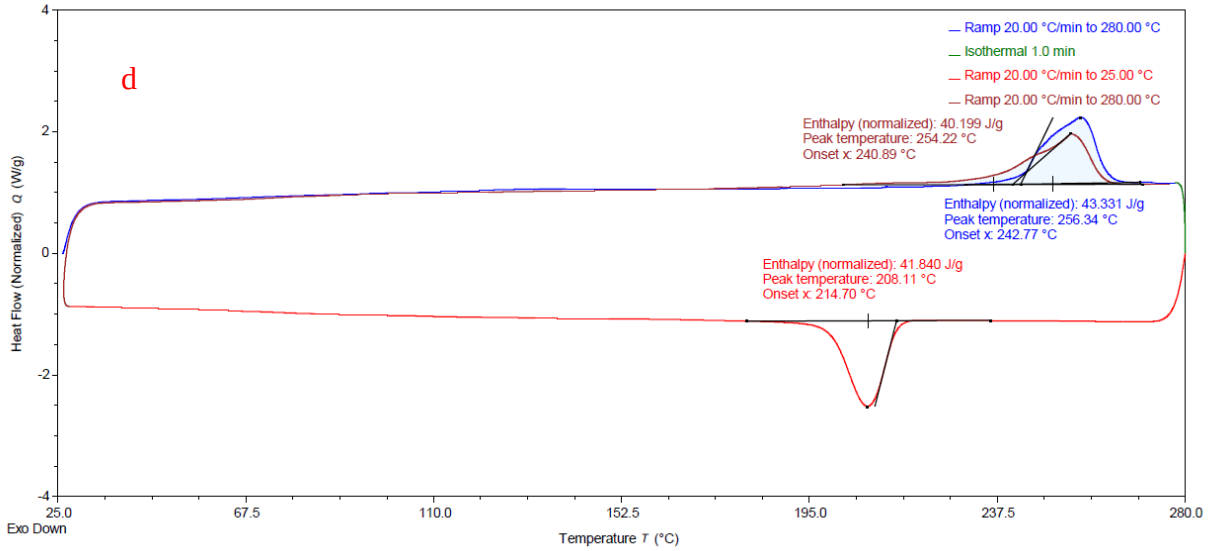


PET Ref




c
Ramp 20.00 °C/min to 280.00 °C
Isothermal 1.0 min
Ramp 20.00 °C/min to 25.00 °C
Ramp 20.00 °C/min to 280.00 °C
Enthalpy (normalized): 17.017 J/g
Peak temperature: 254.22 °C
Onset x: 241.49 °C
Enthalpy (normalized): 41.204 J/g
Peak temperature: 142.67 °C
Onset x: 142.83 °C
Enthalpy (normalized): 25.727 J/g
Peak temperature: 256.41 °C
Onset x: 244.25 °C
Enthalpy (normalized): 22.273 J/g
Peak temperature: 201.97 °C
Onset x: 208.92 °C
Heat Flow (Normalized) Q (W/g)
6
4
2
0
-2
25.0
67.5
110.0
152.5
195.0
237.5
280.0
Exo Down
Temperature T (°C)

JE5


d
Ramp 20.00 °C/min to 280.00 °C
Isothermal 1.0 min
Ramp 20.00 °C/min to 25.00 °C
Ramp 20.00 °C/min to 280.00 °C
Enthalpy (normalized): 40.199 J/g
Peak temperature: 254.22 °C
Onset x: 240.89 °C
Enthalpy (normalized): 43.331 J/g
Peak temperature: 256.34 °C
Onset x: 242.77 °C
Enthalpy (normalized): 41.840 J/g
Peak temperature: 208.11 °C
Onset x: 214.70 °C
Heat Flow (Normalized) Q (W/g)
4
2
0
-2
-4
25.0
67.5
110.0
152.5
195.0
237.5
280.0
Exo Down
Temperature T (°C)

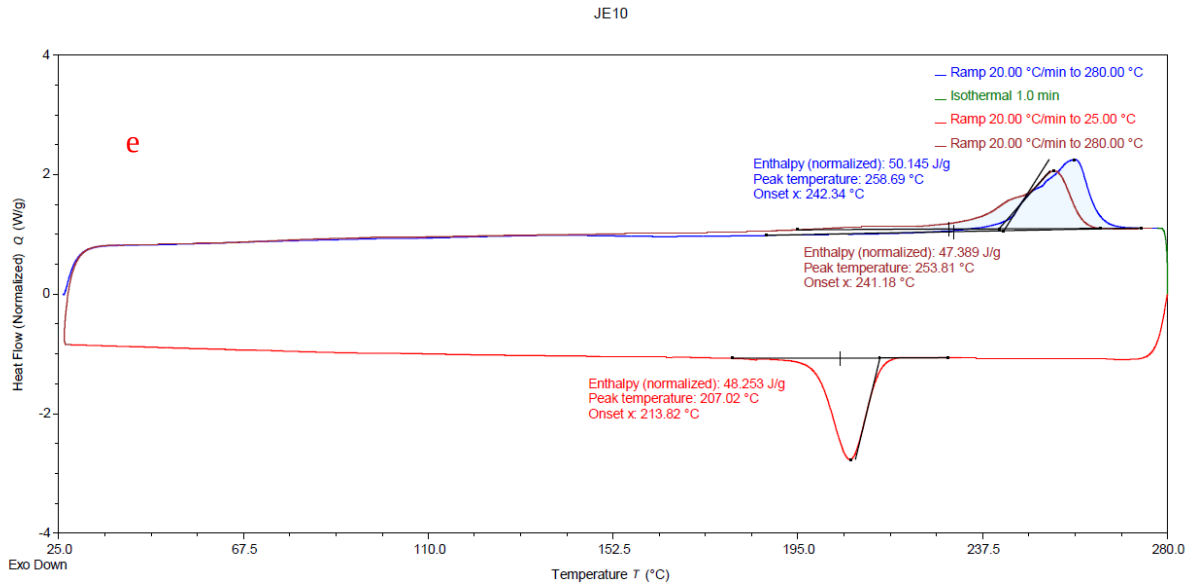


Table 4.6.3. Peak data from DSC heating curves, including melting onset and peak temperatures for samples with polyester content.

		First Heat Melting Peak (°C)			Second Heat Melting Peak (°C)		
Fabric	Peak at 140°C (Y/N)	Onset	Peak	Shoulder (Y/N)	Onset	Peak	Shoulder (Y/N)
Cotton Reference	Y	none	none	N	none	none	N
PET Reference	N	245.67	254.12	Y	233.62	254.03	N
50/50 PET/Cotton	Y	244.25	256.41	Y	241.49	254.22	Y
80% Cotton Removed	N	242.77	256.34	Y	240.89	254.22	Y
PET Enzyme	N	242.34	258.59	Y	241.18	253.81	Y

The no enzyme (control) treated PC fabric (Figure 4.6.3c) sample exhibited all the peaks found in the separate reference 100% cotton and 100% polyester samples, but at lower intensities due to their relatively lower weight percentages in the blended fabric sample. The enzymatically separated polyester (Figure 4.6.3e), had all the peaks observed in the polyester reference sample and had no peak at 140°C, implying an absence of cotton in that sample. The 80% cotton removal sample also showed no peak at 140°C and no major divergence from the onset and peak melting

temperatures from the reference PET (4.6.3d). Because of this behavior suggesting an absence of cotton in the sample, the sensitivity of this analytical methodology to blends was not considered high enough to determine that all of the cotton from a sample is truly removed. Therefore, conclusions from DSC thermograms were primarily drawn from thermal transitions rather than blend composition.

Divergence of the peak melting temperature or melting temperature range from the reference PET would imply a reduction in PET quality because of enzymatic treatment for the enzymatically cleaned PET sample. An example of quality reduction could be PET damage during processing that caused a reduction in molecular weight and consequently, thermal properties. In Table 4.6.3, the onset and peak melting temperatures of both heating cycles for the tested samples are compared. Because the PET reference and the PET/cotton blend fabric come from different sources, some minor variation between their thermal data was expected, which would then be reflected in the enzymatically cleaned PET that originated from the blended fabric.

In the first heating cycle, the reference PET showed onset melting around 245°C and peak melting at 254°C, while the enzymatically cleaned PET showed a broader peak with onset melting at 242°C and peak melting at 258°C (Table 4.6.3). In the second heating, both samples showed earlier onset melting temperatures, with peaks at 254°C. In all cases, the second melting peak was broader than the first, though this was most pronounced in the reference sample. Peak broadening could reflect the processing history of the PET sample. For example, the reference PET was not processed before testing whereas the enzymatically cleaned PET was washed and dried 10 times to simulate the ware seen in a textile waste material before its enzymatic treatment, so the broadened peak could be explained by the fabric history. The same broad peak was observed in

the untreated blend sample, which has the same processing history as the enzyme-treated sample. This correlation suggests the enzyme is not the factor contributing to that peak broadening.

Further, all samples originating from the blended fabric contain a shoulder in both melting peaks, where there is a distinct leveling off before the peak height is reached. A similar shoulder was observed during the first heating of the reference PET. This shouldering effect has been previously observed through DSC analysis of PET after recycling cycles, where the original virgin sample showed no shoulder, and recycled samples showed a more pronounced shoulder over time [45]. It was suggested that this shoulder could have appeared because of contamination, suggesting a reduction in quality. Because all samples that originated from the blended fabric are known to have some residual cotton, it could be concluded that the cotton is causing the shoulder effect. However, a foundational study on the DSC response of cotton/PET blended fabric from 1981 does not show that same shouldering effect in the PET melting peak [73] tested at the same heating rate as the samples in this work. So, cotton contamination may not be the reason for that effect in the tested samples for this work. Rather, it could be that the PET quality in the 50/50 blended fabric is lower than that of the virgin reference sample, which has caused this shoulder in all samples. Regardless, the melting behavior observed between the untreated blend sample and the enzymatically cleaned sample are comparable, suggesting that the enzymatic treatment itself has not caused any quality reduction of the PET fibers.

Through this data, it was concluded that the thermal properties of PET were not reduced because of enzymatic treatment, implying that the PET has not been damaged and should be suitable for the same mechanical recycling or chemical recycling pathways as any other PET sample of the same quality. This result was expected because of the known selectivity of enzymatic

attack, which only degrades the cellulosic component of a substrate, and provides evidence that this methodology is useful for PET isolation from a blend without fiber damage.

5. CONCLUSIONS AND FUTURE WORK

In this study, enzymatic hydrolysis of cotton (or cotton-containing) and viscose fabrics was successfully achieved using cellulase enzymes in a 2mag dry bath mixer. The optimal conditions were investigated for bleached cotton fabric degradation, and it was determined that mechanical agitation was a highly impactful parameter on efficient hydrolysis. Mechanical agitation in this work was carried out using an aggressive beating effect with two magnetic stir bars in each sample. Bleached cotton fabric was successfully degraded into highly crystalline recalcitrant fiber and soluble sugar components at a 4x enzyme dose after 19 hours of enzymatic treatment. Woven viscose fabric was almost entirely converted into soluble components after 45 hours of treatment at a 2x enzyme dose without agitation.

Identified degradation obstacles like reactive dyes, a durable press finish, and blended fabrics were tested through isolation of each variable on prepared waste-simulating fabrics, and the interference that each individually had on enzymatic hydrolysis was investigated. In the case of reactive dyes, the dye slowed the degradation rate because of reduced access of cellulase enzymes to glycosidic linkages. This effect was more prominent for a bifunctional reactive dye, which caused cellulose crosslinking and significantly reduced fabric degradation into a slurry. For example, in the same conditions under which a bleached cotton fabric was fully degraded, a bifunctionally dyed fabric retained 45% large solid fabric pieces. Despite the negative impact on hydrolysis efficiency, both monofunctional and bifunctional dyed cotton fabrics were able to be fully converted into slurry components by cellulase enzymes alone after sufficient reaction time and enzyme addition, without the need for a dye removal step.

A durable press (DP) finish created a greater obstacle than reactive dyes to degradation because of the high level of polymer crosslinking, achieving less than 10% degradation after 19

hours of enzymatic treatment. Chemical pretreatments to mitigate this effect were studied, and a successful chemoenzymatic pathway was implemented. Both acid pretreatment and sequential acid and alkali pretreatment were combined with enzymatic treatment, achieving full DP fabric degradation after 38 hours of enzymatic treatment.

Several blended fabrics were tested, including a bleached 50/50 polyester cotton blend, a dyed 55/45 modacrylic/cotton blend, and a dyed and DP finished 55/45 modacrylic/cotton blend, the latter two fabrics coming from a commercial source. Effective separation of the polycot blend and the dyed modacrylic blend was achieved with sufficiently high enzyme dose after 38 hours of enzymatic treatment. The crosslinking finish on the final fabric was counteracted using the developed chemoenzymatic pathway, achieving 80% cotton removal which was a very positive result for this highly degradation-resistant fabric.

Degradation products including recovered synthetic solids from blended fabrics, recalcitrant cotton fibers, and soluble components were studied. Recovered synthetic solids were shown to be sufficiently separated from cotton through comparison of FTIR, TGA, and DSC data between enzymatically cleaned fibers and a reference synthetic sample, suggesting they should be suitable for mechanical or chemical recycling into new textile fibers. Recovered cotton fine fiber fragments from bleached cotton were shown to have high crystallinity, but fibers recovered from chemically modified fabrics were not as high. Fiber length distribution and reduction over time varied for each fabric type. Fibers retained after separation from a polyester blend showed smaller particle size than bleached 100% cotton fibers. This data supported the theory that separation of cotton fibers from synthetic solids through enzymatic hydrolysis occurs when the fibers have degraded to a small enough particle size that can fall out of the intact synthetic fabric structure. Through LC-CAD analysis, it was determined that glucose made up more than 90% of the detected

soluble sugar components, with all other components from the fabric being disaccharides. The measured glucose content for each sample correlated well with predicted glucose concentrations based on gravimetric analysis, suggesting that gravimetric prediction could be a useful rudimentary way to estimate glucose content for bleached cotton samples.

There are still questions that need to be addressed on enzymatic hydrolysis of fabrics and end use of the degradation products. Physical fabric qualities like yarn and knit structure should be further investigated to better understand the relationship between fabric density and hydrolysis as well as the particle size required for cotton fibers to fall out of blended structures, which could possibly be controlled by the yarn structure. Further, it is important to investigate other common fabric dye classes, like direct and vat dyes, and other common finishes like water-repellency. Soluble degradation products of chemically modified fabrics, including the dyed and finished fabrics investigated in this study, should be identified and quantified as well to understand the potential use for residual syrups after enzymatic treatment given their anticipated complex degradation product matrix.

All generated degradation products should be investigated in the future in different end use pathways. For example, recovered PET solids should be used as feedstock for chemical or mechanical recycling pathways to prove their viability in those circular processing options despite the small amounts of cotton that remain deeply embedded and entangled in the fabric structure. Further, uses for recalcitrant cellulose fragments should be investigated, including use in composites, films, and as feedstock for regenerated cellulosic fiber options. Differences in fiber fragment properties between fabric types used during this study also need to be investigated relative to these recycling pathways. For example, cotton fibers recovered from bleached cotton

fabric have different crystallinity from those recovered from an acid-pretreated DP fabric after enzymatic degradation, so their optimal recycling pathways could be different.

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APPENDICES

Appendix A: Determination of the Standard Hydrolysis Conditions

Several processing decisions were tested as isolated parameters that could be controlled during development of the 2mag degradation method. The parameters included fabric swatch size, sample mass, liquor ratio, time, and mechanical agitation. After repeated observations across tests, sample slot in the 2mag mixer was also tested as a controllable parameter.

Appendix A-1: Effect of Swatch Size on Hydrolysis

Cutting fabrics is known to aid their degradation efficiency by increasing surface area accessible to enzyme attack (see Section 2.2.2). Early on in hydrolysis testing, an experiment was carried out with the 2mag mixing method to test the effect of swatch size on cotton fabric degradation. The selected swatch sizes were 0.5, 1, and 1.5cm^2, with all samples treated in triplicate at a 1x dose. As shown in Figure A-1, smaller swatch size degraded more efficiently than larger swatch size. The difference between large solids remaining from 1 and 1.5cm^2 swatches is prominent, having 4% and 25% on average respectively (Table A-1.1). The difference between 0.5 and 1cm^2 was less significant, despite 0.5cm^2 swatches having no large solids remaining.

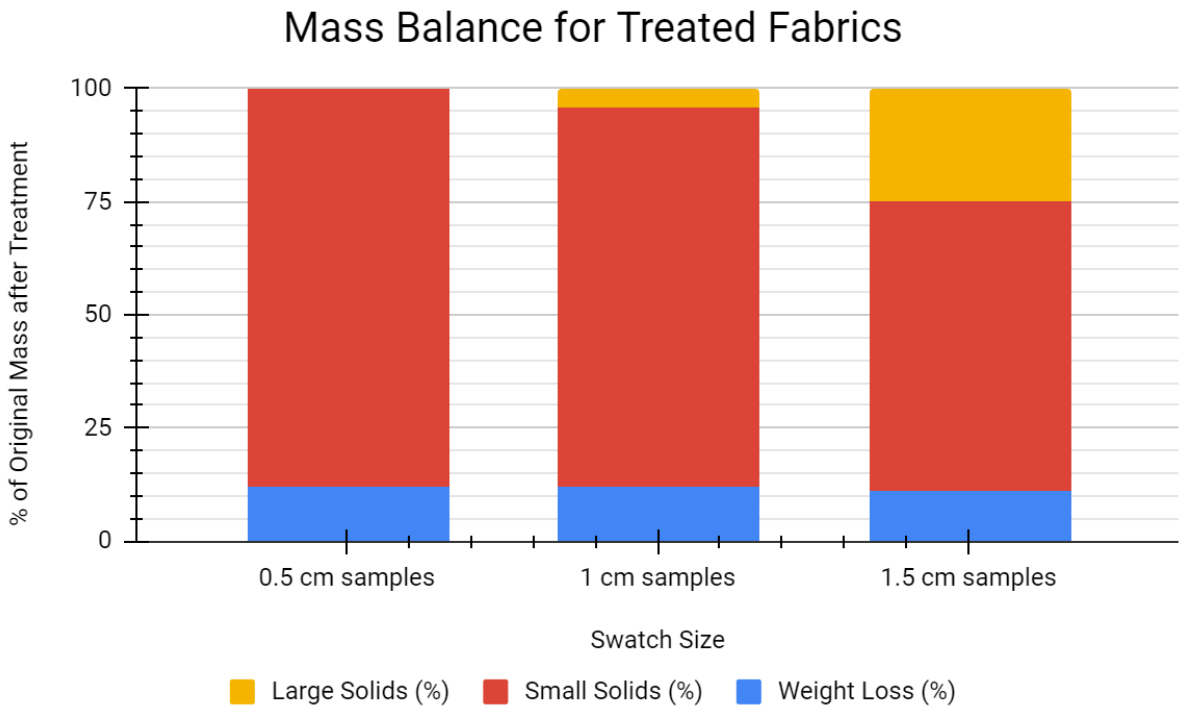

Figure A-1. Gravimetric results from a 1x dose of DC fabric with varying swatch sizes after 19 hours.

Table A-1.1. Average gravimetric data from a 1x dose enzymatic hydrolysis of DC fabric with varied swatch sizes.

Swatch Size	Weight Loss (%)	Small Solids (%)	Large Solids (%)
0.5 cm samples	12.02	87.97	0.00
1 cm samples	11.87	84.00	4.13
1.5 cm samples	11.05	64.09	24.86

Table A-1.2 shows the individual sample results, where JE4 and JE6 with 1cm^2 swatches and had no large solids remaining after treatment. JE5 was the only 1cm^2 sample with large solids remaining, which is due to inconsistent mechanical agitation. This trial was conducted before

machine consistency testing (see Appendix A-6), and JE5 was placed in a sample slot that was later seen to cause reduced agitation. Even without that retrospective conclusion, 1cm^2 sample sizes were chosen as the standard swatch size because of their minimal variation in both weight loss and large solids from the smaller swatch size and because they were more convenient to process than the smaller size. Further, for the samples containing a synthetic component, the larger swatch size was also useful to collect residual large solids of a large enough size for convenient analytical testing in the future.

Table A-1.2. Gravimetric data from individual samples from a 1x enzymatic degradation of DC fabric with different controlled swatch sizes.

Sample	Swatch Size (cm^2)	Weight Loss (%)	Small Solids (%)	Large Solids (%)
JE1	0.5	10.74	89.26	0.00
JE2	0.5	12.74	87.26	0.00
JE3	0.5	12.59	87.41	0.00
JE4	1	10.70	89.30	0.00
JE5	1	12.57	75.05	12.38
JE6	1	12.34	87.66	0.00
JE7	1.5	11.60	69.49	18.91
JE8	1.5	10.37	51.12	38.51
JE9	1.5	11.17	71.63	17.19

Appendix A-2. Effect of Sample Mass on Hydrolysis

To find a balance between sufficiently large sample quantity for convenient gravimetric analysis while achieving efficient degradability of cellulosic fiber content, sample mass was tested as a variable. A 1x dose enzymatic degradation experiment using triplicate KK fabric samples of 0.5, 1, and 2.5 grams was carried out to test this parameter. As sample mass increased, the extent of degradation decreased (Figure A-2). The degradation results from 0.5 and 1g samples were similar, with about 69% and 70% large solids remaining, respectively, while both showed 6.6% weight loss (Table A-2). The decreased hydrolysis of 2.5g samples (~77% residual large solids) was attributed to the mixing dynamics in the 2mag method. Because a larger sample mass required a solids level above the height of the stir bars, the beating mechanical action on each individual sample piece was low compared to samples with smaller mass. So possibly the mass itself was not the limiting factor, but rather it could have been the reduced mechanical action on a larger sample, which in turn reduced hydrolysis. Because there was little difference between 0.5g and 1g samples, the 1g sample amount was chosen as the standard condition for the 2mag method.

Figure A-2. Gravimetric results from a 1x dose hydrolysis of KK fabric at varied sample masses.

Table A-2. Average data for KK fabric samples of different masses treated with a 1x enzyme dose for 19 hours.

Sample Mass (g)	Weight Loss (%)	Small Solids (%)	Large Solids (%)
0.5	6.64	24.84	68.53
1	6.66	23.29	70.05
2.5	6.78	16.13	77.08

Appendix A-3. Effect of Liquor Ratio on Hydrolysis

Liquor ratio is defined as the mass of liquid in the bath to the mass of goods in bath. Textile processes use highly variable liquor ratios, but typically a lower ratio is desirable to reduce water usage. This parameter was tested to determine if liquor ratio influenced hydrolysis and to find the minimum liquid level needed for hydrolysis experiments. Triplicate samples of UC fabric dosed at liquor ratios of 10:1, 20:1, 30:1, and 40:1 were treated for 19 hours with a 2x enzyme dose. Results showed reduced hydrolysis at 10:1 and negligible effects at the three highest liquor ratios (Figure A-3), suggesting this parameter has no bearing on hydrolysis efficiency in this process above a certain threshold. The results for the 10:1 samples were expected because during sample preparation, it was observed that the liquid level was not sufficient to cover the stir bars and fabric pieces in the sample bottle, which is the cause of the reduced hydrolysis. 20:1 was chosen as the standard liquor ratio because it was the minimum level of bath required to cover the fabric and stir bars, leading to efficient hydrolysis that did not increase at other liquid levels (Table A-3).

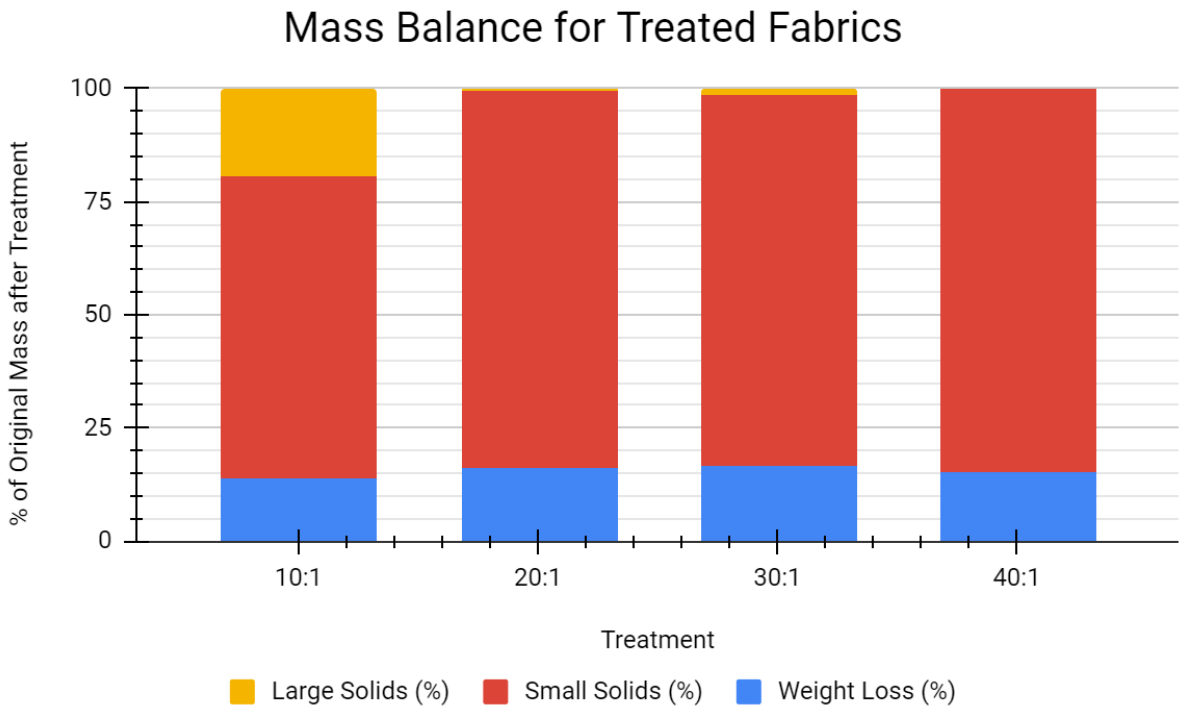

Figure A-3. Gravimetric data from a 2x enzymatic degradation of UC fabric using different liquor ratios.

Table A-3. Average gravimetric data from UC fabric samples treated with a 2x dose at varied liquor ratios.

Liquor Ratio	Weight Loss (%)	Small Solids (%)	Large Solids (%)
10:1	13.92	66.63	19.44
20:1	16.22	83.11	0.67
30:1	16.81	81.72	1.48
40:1	15.29	84.61	0.18

Appendix A-4. Effect of Time on Hydrolysis

Reaction time was expected to increase enzymatic hydrolysis, so was tested as a parameter to determine a convenient and sufficient length. Preliminary experiments were arbitrarily carried out at 19 hours because of convenience, but most data from previous studies is reported after 24 hours in the reviewed literature (see Textile Hydrolysis in Literature Review). To understand the significance of the additional 5 hours of treatment, a study of duplicate KK fabric samples was conducted at different lengths. Predictably, hydrolysis increased with extended incubation time (Figure A-4). While 4 hours was clearly insufficient for total fabric degradation in this study, this parameter offered some insight into degradation kinetics. The 19-hour sample had about 68% large solids remaining while the 24-hour sample had about 63% (Table A-4). Despite this increase, 19 hours was chosen as the standard incubation time because a significant amount of previous testing was conducted at this length because of labor convenience, and it was more beneficial to keep producing comparable data than to redo previous testing at a 24-hour incubation time. Further, the "baseline" UC fabric degradation was completely achieved after 19 hours and did not require extension to 24 (see UC Hydrolysis in Results), so it made sense to use this as a standard incubation length.

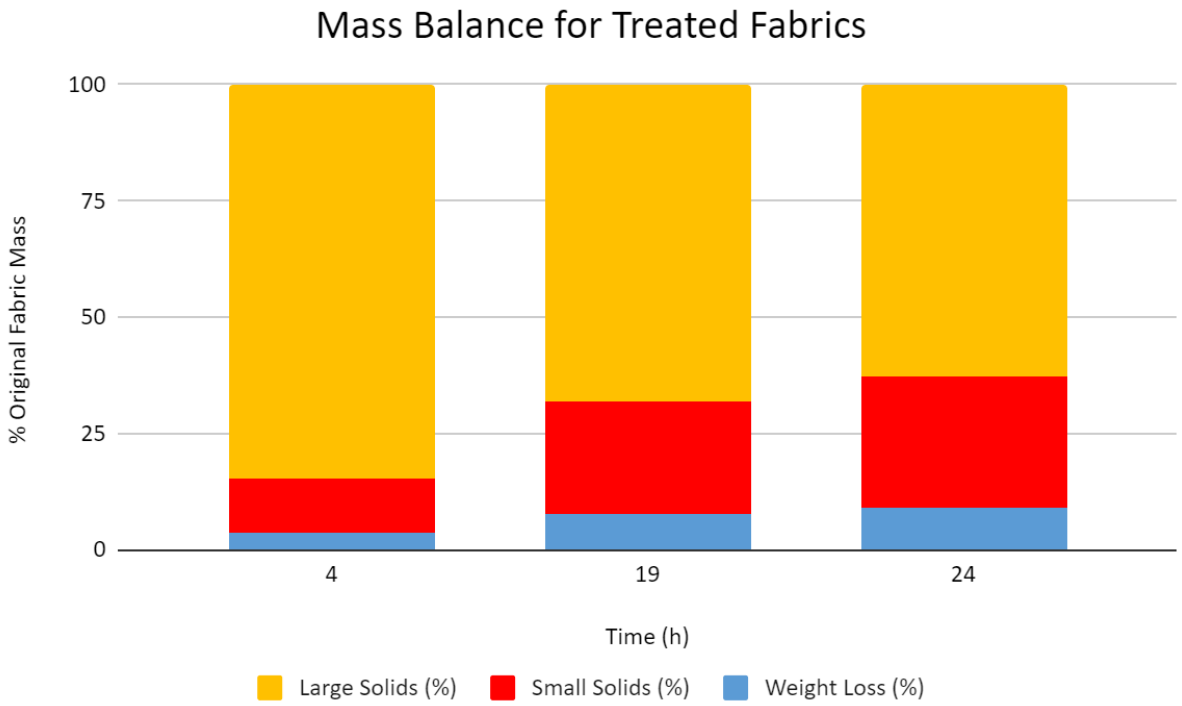

Figure A-4. Gravimetric data from varied incubation length enzymatic degradation experiments using a 1x dose on KK fabric.

Table A-4. Average gravimetric data for 1x dose enzymatic treatment of KK fabric at varied incubation times.

Time (h)	Weight Loss (%)	Small Solids (%)	Large Solids (%)
4	3.80	11.56	84.64
19	7.64	24.11	68.26
24	8.81	28.45	62.74

Appendix A-5. Effect of Mechanical Agitation on Hydrolysis

Mechanical agitation has been previously observed to be an important parameter in enzymatic hydrolysis (see Textile Hydrolysis in Literature Review). To determine an efficient

mechanical agitation method using the 2mag mixer, a 2x dose enzymatic degradation study was conducted using triplicate samples of BC fabric either with no stir bars (control), 1 stir bar, or 2 stir bars in each sample bottle to test the effect of common “stirring” agitation versus the “beating” effect reported by Vasconcelos et al. [31]. It was shown that addition of stir bars increased hydrolysis efficiency (Figure A-5). Large solids remaining reduced from 86% to 35% to 2% with the addition of a stir bar (Table A-5). Interestingly, the sample with no stir bars showed a higher proportion of glucose-to-small-solids than the samples with agitation. This demonstrated the importance of agitation in disintegration from fabric into fiber.

Notably, single stir bar addition also provided the desired beating effect at the beginning of incubation, where the fabric swatches interfered with circular stirring motion, which caused the stir bar to jump around. At some point during the incubation period, the large solids were reduced to a small enough size that the singular stir bar was able to carry out its design-intended stirring motion. After this point, the accelerated disintegration was either reduced or eliminated. The addition of the second stir bar counteracted that because the stir bars themselves prevented each other from eventually reaching that circular stirring motion, so they were able to continually beat fabric pieces and fibers throughout the incubation. For this reason, two stir bars was chosen as the standard mixing condition that allowed cotton to be degraded as efficiently as possible.

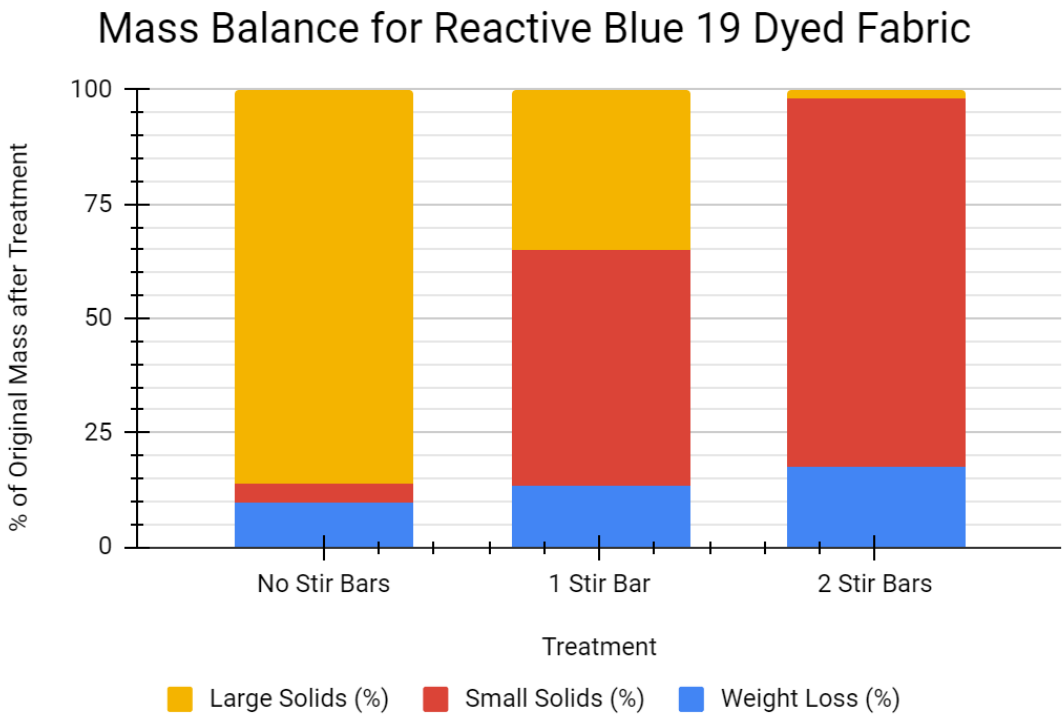

Figure A-5. Gravimetric data from 2x dose hydrolysis of BC fabric after 19 hours with varied mechanical agitation conditions.

Table A-5. Average gravimetric data from enzymatic hydrolysis of BC fabric using a 2x enzyme dose after 19 hours with different mechanical agitation conditions.

Treatment	Weight Loss (%)	Small Solids (%)	Large Solids (%)
No Stir Bars	9.61	4.39	86.00
1 Stir Bar	13.34	51.78	34.88
2 Stir Bars	17.39	80.66	1.95

Appendix A-6. Effect of Sample Slot on Hydrolysis

The 2mag mixer contains 15 sample slots in a 3x5 grid, which are all equally heated and have individual magnetic mixing controls. In early experiments, sample bottles were placed in the mixer in order according to sample number (e.g., samples 1-3 were placed in row 1 and samples 4-6 were placed in row 2, etc., see Table A-6). Across experiments, it was observed that sample bottles in middle slots were consistently lower than those on the outside (i.e., sample 5 would consistently show less degradation than sample 6 when treated at the same conditions). Of course, sample variation is expected, but it seemed unlikely that the sample showing lower degradation would always be the same number. To test this, 15 UC samples were tested at a 1x dose simultaneously to see if any effects were observed based on sample position. As expected, there was variation across all individual samples (Figure A-6.1). While none of this variation is statistically significant (see blue box plot in Figure A-6.2), it was seen that the "middle" samples, being those in the middle slot of rows 2-4 (samples 5, 8, and 11, see Table A-6) showed the least degradation of all the samples. Samples on the outer sample slots, being those that have an "exposed" side showed higher degradation. This behavior caused for a more extended range below the median than the range above the median in the generated box plot (Figure A-6.2), and much tighter grouping of sample data is observed when those three middle samples are removed from the data.

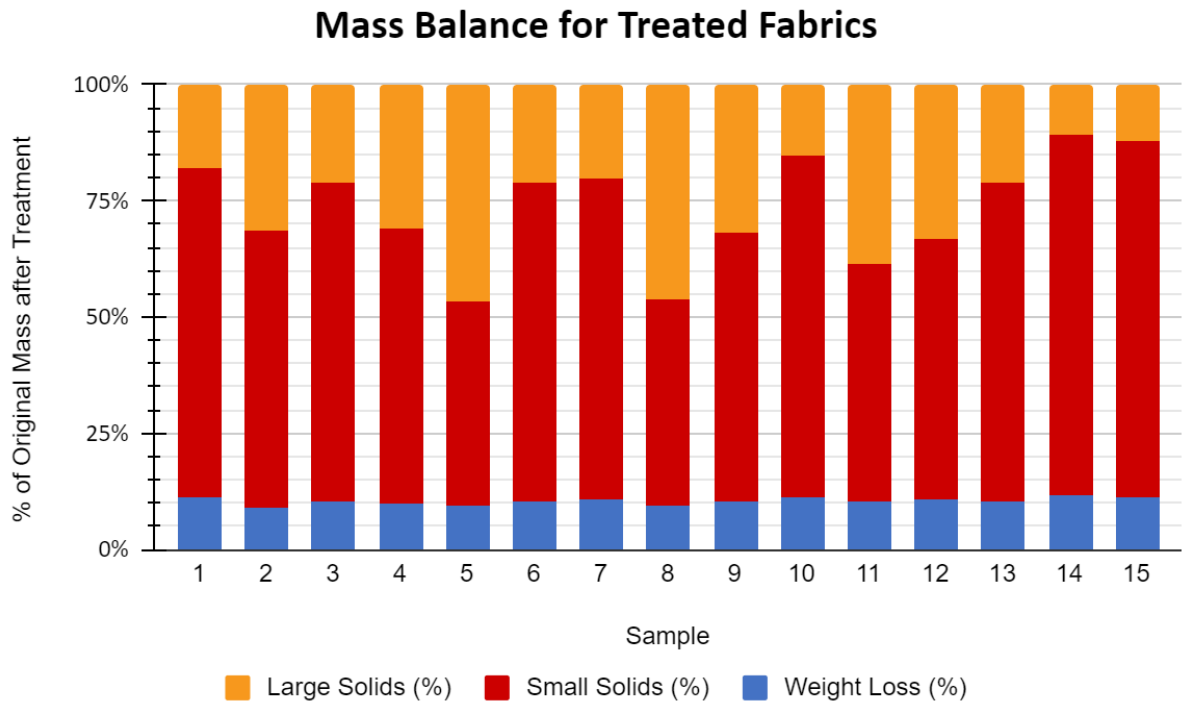

Figure A-6.1. Individual gravimetric sample data from a 1x degradation of UC fabric after 19 hours, where the sample number corresponds with the assigned sample slot in the 2mag mixer.

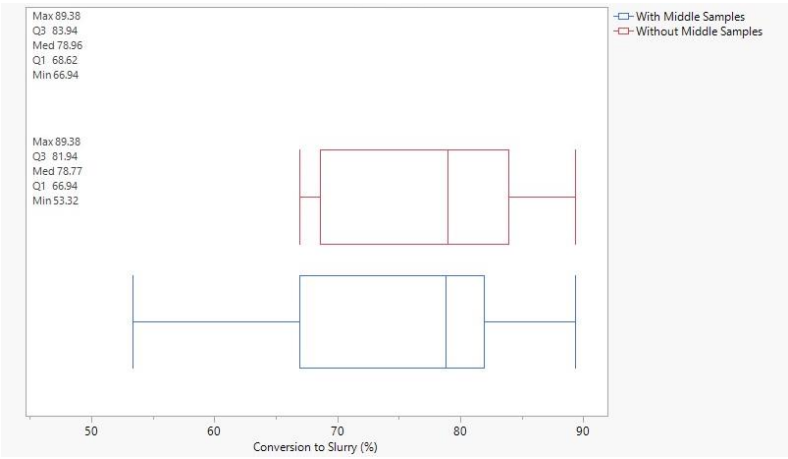

Figure A-6.2. Box plots of conversion to slurry for each individual sample in the machine consistency test including samples in middle slots and excluding samples in middle slots.

Table A-6. Schematic layout of 2mag mixer samples slots with row and sample numbers defined, where each numbered cell corresponds to a sample slot.

	Sample Number		
Row 5	13	14	15
Row 4	10	11	12
Row 3	7	8	9
Row 2	4	5	6
Row 1	1	2	3

Sample 12 also showed lower degradation than other outer slots, but this was concluded to simply be sample variation because of agitation inconsistency. The same was not concluded for the middle slots because of the repeated behavior observed across multiple trials. It was concluded that the abnormal agitation with 2 stir bars caused interference between samples, as this machine is designed for consistent mixing using 1 stir bar. Any sample bottle surrounded on all sides by other actively mixing samples seemed to receive less of the desired “beating” effect than other samples. Because of this, most future trials were run with a maximum of 12 samples around the outside of the machine, to avoid this effect from negatively influencing the average data for a given sample set in studies.

Appendix B: 0.5 DP Hydrolysis

While most experiments concerning DP finished fabrics were carried out on the standard dose fabric, a subset of testing was carried out on the half dose (0.5DP). After enzymatic treatment at standard conditions, 0.5DP fabric showed more degradation than DP fabric (Figure B.1 and Figure 4.3.32), with a 4x dose achieving 16% degradation, where the DP fabric achieved less than

10% (Table B.1 and Table 4.3.3.1). This was expected because of the lower level of crosslinking applied to the fabric, which allowed more access of the cellulase enzymes to glycosidic attack.

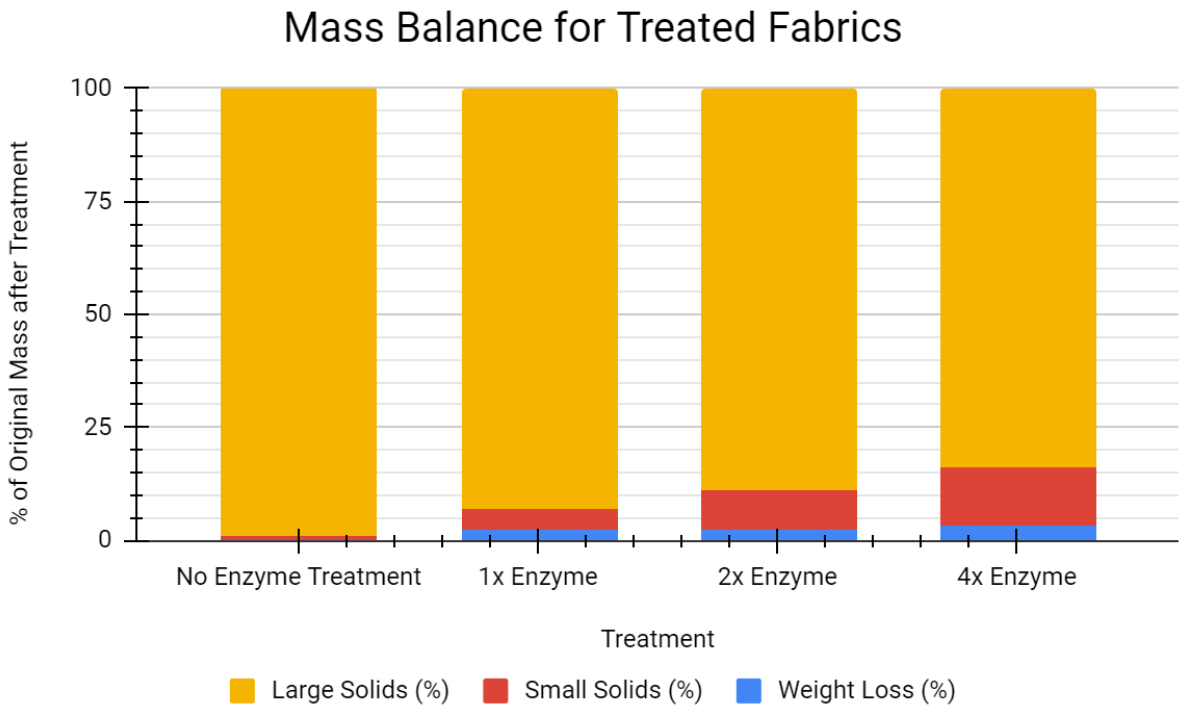

Figure B.1. Gravimetric results for degradation of 0.5DP fabric at standard conditions.

Table B.1. Average results for the degradation of 0.5DP fabric treated at standard enzyme conditions.

Treatment	Weight Loss (%)	Small Solids (%)	Large Solids (%)
No Enzyme Treatment	-0.40	0.82	99.58
1x Enzyme	2.23	4.70	93.07
2x Enzyme	2.52	8.36	89.12
4x Enzyme	3.13	12.85	84.02

After pretreatment with cold alkali, 0.5DP degradation improved significantly (Figure B.2). Degradation after 19 hours of a 4x dose improved from 16% to 56% (Table B.2), which is more degradation improvement than observed after cold alkali pretreatment of DP fabric. Where DP degradation increased by 167% because of cold alkali pretreatment (from 7.5% to 20% degradation), 0.5DP degradation increased by 250% (Figure B.2). It is reasonable to conclude that the reduced crosslinking allowed for more significant fiber swelling and crystal disruption in the half dose than the full dose DP fabric. These combined effects would explain the more greatly improved hydrolysis in the 0.5DP than the DP fabric.

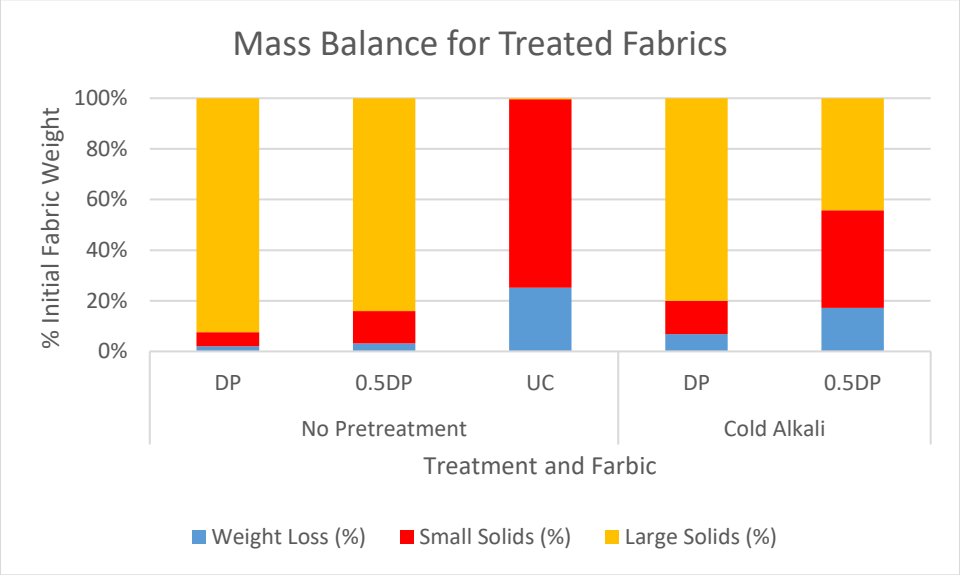

Figure B.2. Comparison between gravimetric results for 0.5DP and DP fabrics with and without chemical pretreatment, enzymatically treated at a 4x dose. UC degradation was added as a comparison to the baseline achievable degradation for unfinished cotton.

Table B.2. Average gravimetric results for 0.5DP, DP, and UC fabric with and without chemical pretreatment, followed by a 4x 19-hour enzyme treatment.

Pretreatment	Fabric (4x Dose)	Weight Loss (%)	Small Solids (%)	Large Solids (%)
No Pretreatment	DP	2.06	5.53	92.42
	0.5DP	3.13	12.85	84.02
	UC	24.92	73.99	0.38
Cold Alkali	DP	6.79	13.19	80.02
	0.5DP	17.25	38.54	44.21