Recycling Mattress Shoddy by Enzymatic Depolymerization of PET and Cotton to Monomers for Value-Added Applications

FINAL REPORT

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Executive Summary

This study explores the technical feasibility of recycling post-consumer mattress shoddy felt textile pads using enzymatic catalysis to convert its components into building block chemicals. Shoddy is commonly used as a layer to separate metal springs from foam comfort layers in mattress construction. The shoddy pad is typically a non-woven blend of cotton and polyester textile fibers. Other natural or synthetic fibers such as wool, camel hair, rayon, polypropylene, and recycled fabric scraps may also be used. Due to its variable and heterogeneous composition, attempts to mechanically recycle shoddy have been largely unsuccessful. In mattress recycling operations, almost all the recovered shoddy is simply discarded and sent to landfill. According to the Mattress Recycling Council, which sponsored this study, shoddy represents approximately 10% of non-recyclable product sent to landfill.

Compared to conventional chemical and thermal processes, enzymatic catalysis is a low energy intensive and highly selective method to recover monomers which may enable reuse in new polymers or other high value applications. Polyesters, such as polyethylene terephthalate (PET), are degradable by cutinases. Cotton, composed of cellulose, are degradable by cellulases. Due to their innate selectivity, these enzymes can be used to target specific components of mixed fiber materials.

The research team was able to demonstrate a one-pot, two-step process to separately depolymerize cotton to glucose and PET to terephthalic acid in a post-consumer shoddy pad with a 60/40 PET/cotton composition. Reactions with each component of the blend could be carried independently without undesired side reactions with the other substrate. Unfortunately, yields were rather low. Starting with cellulase, approximately 20% of the cotton was reduced to glucose. High crystalline content in the PET component was found to inhibit the cutinase depolymerization reaction. Only 5% conversion of PET to terephthalic acid was observed. Steps to reduce crystallinity through a melt quenching procedure did not improve conversion yields.

Further work is required. It is believed that further preparation of fiber material to sizes less than 1 mm and continuous agitation of the reactor mix, done to improve fiber wetting, will improve enzyme engagement. Filtration techniques to minimize fiber loss are also expected to improve yields. Lastly, utilizing more current generations of engineered enzymes is expected to improve results.

Introduction

The work described herein details the results of a research project focused on utilizing enzymatic catalysis to depolymerize polyethylene terephthalate (PET) and cellulose polymers, which make up the chemical structure of fibers contained in mattress shoddy pads. The Mattress Recycling Council (MRC) funded this work, enabling the acquisition of a Fritsch "Pulverisette 11" knife mill, equipment and chemicals and stipends for two undergraduate students to directly develop and complete experiments for this work. The results of the work are summarized in section two of this report. Materials and detailed procedures for experiments conducted are provided in section three. Supplemental figures are provided in section four.

1. Research Objective

In this work, the PET degrading enzyme ***Humilica insolens* cutinase (HiC)** (Novozym® 51032) and cellulose degrading, engineered cellulase-cocktail designed by Novozym®, **Cellic CTec3HS**, were investigated as agents for the degradation of PET and cellulose in shoddy mattress pads. The amount of monomer released from each polymer (PET – terephthalic acid, cellulose – glucose) was quantified. An enzyme cascade protocol was investigated to develop a one-pot, two-step, fiber degradation procedure.

2. Discussion of Results

2.1 Determination of Polymer Composition and Thermal Analysis of Shoddy Pad

A shoddy pad, provided by the Mattress Recycling Council (MRC) and denoted as sample # 12, was chosen due to the observed heterogeneity of fibers, suitable size for handling, and assumed binary polymer mixture. Other shoddy pads provided by the MRC contained nylon which was not a polymer of focus for this study.

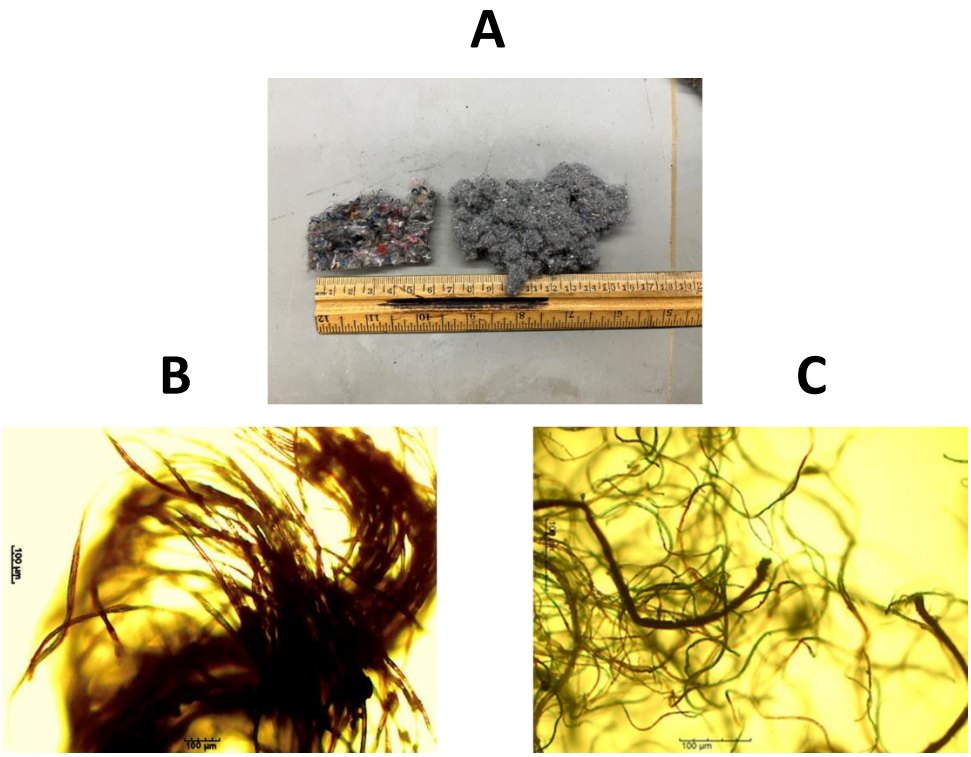

Figure 1. **A.** Shoddy sample # 12 before and after shredding. **B.** Optical microscopy image (4x magnification) of shoddy sample # 12 before shredding and **C.** after shredding.

Shoddy sample # 12 was cut into 5.0 x 5.0 cm squares and shredded into homogenous fiber mixture using a Fritsch Pulverisette 11 knife mill. An image of pre- and post-shredded shoddy sample # 12 is provided in **Figure 1.** Using optical microscopy to view the structure of the fibers in shoddy sample # 12, it was observed that shredding the shoddy pad decreased the thickness of woven fibers. The observed decrease of woven fiber thickness is beneficial, such that the surface area of the polymeric substrate is increased for better enzyme adsorption. The shredded shoddy sample # 12 was used for all experiments described herein.

The polymer composition of the shredded shoddy sample was first evaluated using ATR-FTIR spectroscopy (See appendix for figures). The carbonyl (C=O) of PET was observed at 1700 cm^{-1}, whereas the hydroxyl (-OH) functional group of cellulose was observed at 3320 cm^{-1}. The observed vibrational modes correlate to pure samples of PET and cellulose. Thus, it was concluded that shoddy sample # 12 is a binary mixture of PET/cellulose. The %-composition of the binary polymer mixture was determined using ASTM-D629-15, which utilizes 70% sulfuric $acid_{(aq)}$ to dissolve cellulosic components within a polyester/cellulose mixture. After cellulose dissolution, the remaining material (undissolved polyester) is recovered, dried, and weighed. Thus, the %-ratio of polyester to cellulose within the fiber sample can easily be calculated based on mass difference

before and after 70% sulfuric acid dissolution. The content of PET in shoddy sample # 12 was determined to be 59.7 ± 2.1%, whereas cellulose accounted for the other 40.3 ± 2.1%.

Differential Scanning Calorimetry (DSC) was used to estimate the crystallinity of the PET fraction of shoddy sample # 12 (See appendix for figures). Analysis of shoddy sample # 12 via DSC resulted in a heating curve with three melting peaks at 127.3, 165.9, and 252.57 °C. The temperatures observed at 127.3 °C and 252.57 °C correlate to the cold crystallization and melting temperatures of PET, however, the cold-crystallization peak is negative suggesting the amorphous regions of PET in the shoddy sample # 12 do not recrystallize post-glass transition temperature (75 °C). Integration of the area under the melting temperature resulted in an enthalpy value of 32.69 J/g. Crystallinity (x_c) of the PET polymer was estimated to be 23.6%, which is expected for PET fibers. A reference sample of pure PET was analyzed using DSC to compare to shoddy sample # 12. The glass transition, cold crystallization, and melting temperature of pure PET are observed on the heating curve at 76.7, 127.94, and 246.83 °C. Comparison of the melting temperatures of pure PET with the melting temperature of shoddy sample # 12, upon a 2nd heat-cool cycle, confirms the identity of PET in the shoddy pad, albeit the melting temperature of pure PET is lower than the expected melting temperature. This occurrence is most likely due to the significant difference in crystallinity between the PET reference (5.0%) and shoddy sample # 12 (23.6%). A reference sample of pure cotton was analyzed using DSC for comparison purposes, however, cellulose does not possess a glass transition or melting temperature.

2.2 Enzymatic Hydrolysis Experiments

2.2.1 Alkaline vs. Aqueous Pretreatment for Cellulase Hydrolysis

Two reported pretreatments for cellulose-based materials, cold NaOH/Urea and water reflux, were compared against a control sample of shoddy sample # 12 to evaluate whether basic or water-based pretreatments would improve glucose liberation from cellulose polymers in the shoddy sample. Based on mass differences before and after pretreatment, it is apparent that some mass is lost most likely due to small fibers passing through the sieve. After cellulose hydrolysis, the alkaline pretreatment, water reflux pretreatment, and control experiment yielded 58.1 ± 0.21 mg, 58.5 ± 0.8 mg, and 60.9 ± 1.4 mg of shoddy, respectively. Therefore, future experiments did not perform any chemical pretreatments on shoddy sample # 12 prior to enzyme hydrolysis.

2.2.2 Quantification of Liberated Glucose Concentration

For all cellulase-based hydrolysis experiments, monomeric glucose was liberated from cellulose chains within the cotton fibers of 100.0 mg of shoddy sample # 12 using 2.7 mL (100 FPU/mL) of Cellic CTec3HS in 7.3 mL of 50 mM citrate buffer (pH 4) at 50 °C for a total of 96 hours inside a sealed 4 Dram (14.8 mL) borosilicate vial. Every 24 hours, a 0.5 mL aliquot was taken from each replicate and the glucose concentration was determined using the Amplex Red Assay kit. On average, after 24 hours, about 11.6 ± 1.2 mg of glucose monomer was liberated via Cellic CTec3HS hydrolysis. The average amount of glucose increased gradually every 24 hours, plateauing at 72 hours, such that an average of 17.2 ± 1.4 mg of glucose in total is liberated from 100 mg of shoddy sample # 12. Given that cellulose comprises about 40% of the shoddy sample # 12 and based on our experimental set up, about half of the expected mass of glucose is absent. We hypothesize that the mass discrepancy is due to fibers being washed through the 150-micron sieve.

2.2.3 Quantification of Liberated Terephthalic Acid and Melt-Quench Pretreatment to Reduce PET Crystallinity

The enzyme *Humilica insolens* Cutinase (HiC) was employed as the catalyst for the depolymerization of PET fibers in shoddy sample # 12. A UV-Vis assay was developed to quantify the concentration of terephthalic acid based on the absorbance of the hydrolysis aliquots measured at 240 nm. Initial experiments resulted in negligible terephthalic acid liberation. It is well established that highly crystalline PET is resistant to cutinase degradation. Therefore, we opted for a melt-quench protocol in which the shoddy sample # 12 was incubated at a temperature greater than the melting point (260 °C) for about 10 mins. Immediately following the incubation time, the shoddy sample was submerged in liquid nitrogen to trap PET polymers in their amorphous state by preventing the recrystallization of PET allotted by slow cooling. Initial DSC analysis revealed that the crystallinity of the PET in shoddy sample # 12 was greater than 20%. After carrying out the melt-quench, the crystallinity of PET was reduced to 1.8%. Therefore, shoddy sample # 12 was subjected to melt-quench prior to hydrolysis via HiC. To our dismay, the melt-quench protocol did not improve terephthalic acid liberation from shoddy sample # 12. After 96 hours of incubation at 80 °C in 50 mM citrate buffer (pH 8) with HiC (113 U/mL), an average of 2.86 ± 0.5 mg of terephthalic acid was quantified in the supernatant of the hydrolysis reaction.

2.2.4 Enzyme Cascade Experiments

In this work, a protocol was proposed that uses an enzyme-cascade in which one enzyme would be added to the reaction mixture following an initial incubation time with the other enzyme. Therefore, a one-pot, two-step depolymerization of PET and cellulose polymers in shoddy pads was probed. Based on our previous experience with poor PET depolymerization, we opted to investigate the development of the cascade beginning with cellulase, followed by cutinase addition. Our initial determination of liberated glucose from cellulase-cutinase enzyme cascade experiments revealed that the cutinase enzyme negatively affects the chosen glucose assay, yielding skewed results. Therefore, isolation of the enzymes using membrane filtration was amended into the protocol. Beginning with cellulase, after 96 hours, a total of 14.1 ± 0.83 mg of glucose was released from 100 mg of melt-quench pretreated shoddy sample # 12. After 96 hours, the pH was adjusted to 8, the temperature was increased from 50 to 80°C, and 113 U/mL of HiC was added to the reaction. Quantification of liberated terephthalic acid revealed an average of 4.62 ± 0.21 mg of terephthalic acid was released.

2.2.5 Conclusions and Recommendations for Future Work

Overall, we have demonstrated that shoddy mattress pads composed of a binary mixture of PET and cellulose fibers are susceptible to degradation via cutinase (*Humilica insolens*) or cellulase (Cellic CTec3HS). To homogenize the fiber composition of shoddy pads (shoddy sample # 12), a shredding protocol was developed to simulate current industrial shredding practices. The composition of shoddy sample # 12 was determined to be 60/40 PET to cellulose based on ASTM-D629-15, and the identity of each polymer in the shoddy sample was confirmed using FTIR and DSC. Overall, using 100.0 mg of shoddy sample # 12, the quantity of glucose released did not exceed 20.0 mg and the quantity of PET liberated did not exceed 5 mg under our experimental conditions.

Highly crystalline PET is known to be a poor substrate for HiC degradation. Therefore, a melt-quench protocol was attempted to lower the crystallinity of the PET fibers within the shoddy sample. Prior to melt-quench, the crystallinity was determined to be greater than 20% and after the protocol was carried out, the crystallinity had decreased significantly to about 2%. Unfortunately, the melt-quench protocol did not result in a dramatic increase in terephthalic acid. The maximum amount of terephthalic acid released did not exceed 5.0 mg. Significantly improved cutinases are

under development which show potential to improve performance in highly crystalline PET fibers. Further investigation of these next generation cutinases is recommended.

We hypothesize that the factors beyond the enzymatic catalysis also play a role in maximizing depolymerization of PET and cellulose fibers. For example, we utilized 4-dram vials with magnetic stirring as the reaction vessel. Some shoddy samples were observed to float in the reaction media and maintained a clumped morphology, which could inhibit the enzyme from engaging the substrate. We recommend a reaction vessel which more aggressively agitates the shoddy pad sample directly during hydrolysis to maximize substrate-enzyme contact. We also believe that further grinding of the fibers is necessary, preferably to less than 1 mm. The resulting increased surface area should speed diffusion and enable increased solids in the reactor mixture thereby enhancing productivity and yields. In addition, some fiber loss was observed during washing steps. Better filtration methods would be needed to minimize fiber loss which would mitigate contamination of washing solvents and loss of valuable feedstocks for glucose, terephthalic acid, and ethylene glycol.

3 Materials and Methods

3.1 Enzymes and Chemicals

Enzyme ***Humilica insolens*** **Cutinase (HiC**) was purchased from Strem Chemicals. The cellulase mixture, **Cellic CTec3HS** was generously gifted by Novozym®. Both enzymes were received as suspensions and used without further purification. Several shoddy mattress pad samples were kindly provided by the Mattress Recycling Council. Sodium Bicarbonate, Urea, and Glycerol were procured from Fisher Chemical. ACS Grade Sulfuric Acid 95-98% was sourced from VWR Chemicals. Ethylene glycol, sodium metabisulfite, phenol, citric acid, tris(hydroxymethyl)-aminomethane (99+%), sodium potassium tartrate, terephthalic acid (98%), polyethylene terephthalate, 3,5-Dinitrosalicylic acid, and D–Glucose were purchased from Sigma-Aldrich. Maleic anhydride and sodium carbonate were obtained from Fluka Analytical and Alfa Aesar, respectively. All other chemicals were purchased from Thermo-Fischer and used without further purification. The following buffers and solutions were created in the lab: 70% sulfuric acid, 2% sodium bicarbonate, 0.05M citrate buffer, 1M citrate buffer, 0.1M sodium hydroxide (aq.) and 0.5mM tris hydrochloric acid buffer with 10% glycerol.

3.2 Shredding Protocol for Shoddy Pad

Shoddy pad sample # 12 was first shredded using a Fritsch Pulverisette 11 knife mill. The following protocol was developed using a careful, incremental increase to speed and shredding time. First, 3.0 g of shoddy sample # 12, cut roughly into 2.0 x 2.0 cm squares, was added to the chamber of the knife mill. The Vario-lid and sample pusher accessories were used in combination to compress the shoddy squares as the knife mill was engaged for 360 secs at 10,000 rpm. The resultant shredded fiber was transferred to a sealable plastic bag.

3.3 Determination of Shoddy Sample # 12 Polymer Composition

The composition of the shoddy mattress pad sample # 12 was determined using the American Society for Testing and Materials (ASTM) D629-15 Chemical Method No. 5 – Wool or Polyester Mixed with Cellulosic Fibers or Silk. First, cellulose fibers in the shredded shoddy mattress pad sample (100.0 mg) were dissolved in 100.0 mL 70.0% sulfuric acid for 30 mins at room temperature with gentle mixing. The remaining solid was isolated via filtration using 150 μm steel-mesh sieve. The sample was submerged in a beaker of 2% NaHCO3 for five minutes and then washed with 300 mL of water. The residual solid was collected, blotted dry, and placed in an oven at 110 ºC overnight. The solid was taken out of the oven and left to cool in a desiccator for 30 mins prior to recording the final mass. The percent PET within the shoddy sample is equivalent to the mass of the remaining solid (60 mg = 60%), whereas the percent cellulose is equivalent to the mass loss post-sulfuric acid (40 mg = 40%). Experiments were performed in triplicate.

3.4 Crystallinity Determination of PET in Shoddy Sample # 12 via Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) experiments were conducted using 5.0 mg of shredded shoddy fiber placed into a hermetically sealed aluminum pan. The samples were analyzed using a TA DSC 2000 by a heat-cool-heat cycle in the temperature range of 0 – 300°C. The degree of crystallinity (X_c) of an untreated, lyophilized, shredded, shoddy sample # 12 composed of 59.7 ± 2.1% PET, was estimated using the TA Universal Analysis software based on the first heating scan from 0 – 300°C at a rate of 10°C/min, according to the equation:

$$X\mathrm{c} = \frac{\Delta \mathrm{Hf} - \Delta \mathrm{Hc}}{\Delta \mathrm{H°f}}$$

where ΔHf is the enthalpy of fusion determined by the integration of the endothermic melting peak using a straight baseline drawn between the visually determined melting start and end points, ΔHc is the enthalpy of exothermic cold crystallization peak and ΔH°f is the enthalpy of fusion of pure 100% crystalline PET polymer (140.0 J/g·°C) at melting temperature (260.0 °C).

3.5 Enzyme Characterization

3.5.1 Enzyme Concentration

As previously mentioned, this research project was performed using a cutinase enzyme (HiC) and a cellulase enzyme mixture (CTechHS3). A NanoDrop One Microvolume UV-Vis Spectrophotometer was used to roughly estimate the concentrations for the cutinase (24.0 mg $mL^{-1)}$ and cellulase (4.9 mg mL^{-1}) enzymes. Water was used as a blank due to the unknown composition of the enzyme suspension solution.

3.5.2 Cutinase (HiC) Activity Assay

Enzyme activity for HiC cutinase was determined using a cutinase-catalyzed PET hydrolysis methodology developed by the Gross Lab. The pH-stat apparatus used was a Titrando 842 with Tiamo 1.1 software. Approximately 15 PET films (crystallinity 7-10%), cut into 5.0 x 5.0 mm^2 circles, were placed in a 50.0 mL polyethylene centrifuge tube filled with 10.0 mL of 0.5 mM Tris Buffer with 10% (v/v) glycerol. The mixture was stirred continuously and kept at 80.0°C. From the stock enzyme solution, 50.0 µL of HiC enzyme was added directly to the 10.0 mL mixture. The mixture was incubated for 1 hour. The total volume of titrated sodium hydroxide (NaOH) to maintain a pH of 8.0 for 60 minutes was measured and cutinase activity is expressed as mmols of NaOH/mL of enzyme/min. The activity for the stock solution HiC enzyme (24.0 mg mL^{-1}) was determined to be 11, 390 U mL^{-1} (1 Unit = umole NaOH/min). The assay was performed in duplicate.

3.5.3 Cellulase Activity Assay

A 96-well microplate cellulase activity assay was developed. To quantify the cellulase activity, a series of enzyme dilutions were performed to discover the specific cellulase concentration that released 2.0 mg of glucose from the Whatman No. 1 filter paper. Glucose

standards (0, 2, 4, 6, 8, 10, 15, and 30 mg/mL) were prepared in 50 mM sodium citrate buffer (pH 4.8). Using a 96-well microplate, 20.0 uL of glucose standards and diluted enzyme solutions were mixed with 40.0 uL of 50 mM sodium citrate buffer (pH: 4.8) in each well. In addition, 5.0 x 5.0 mm^2 circles of Whatman No. 1 filter paper was placed in the enzyme-filled wells. After incubation in a 50.0 ^oC Eppendorf Thermostat Microplate Heater for 1 hour, 120.0 uL of DNS was added to each well and the plate was heated to 95.0 ^oC for 5 minutes. Ina new microplate, 36.0 uL of each reaction was diluted with 160.0 uL diH2O. The absorbance at 540nm was measured. Cellulase activity is reported as filter paper units per mL (FPU/mL). It was determined that a 1:5000 dilution of cellulase enzyme (i.e., 0.0009 mg/mL concentration) would elicit 2.0 mg of glucose from the filter paper. Using **Equation 2**, the activity of the cellulase mixture was determined to be 10,278.9 FPU/mL.

$$F.P.U. = \frac{9.251}{[Enzyme]\ releasing\ 2.0\ mg\ glucose} units/mL$$

3.6 Hydrolysis Experiments

3.6.1 Comparison of Pretreatment Methods for Cellulase Hydrolysis Experiments

In this experiment, three separate experiments were run to determine the difference in the amount of glucose liberated between three different pretreatment methods: NaOH/Urea, Water Heat Reflux, and no pretreatment.

- NaOH/Urea method: 100 mg of shoddy was placed in 10 mL of 7 % (w/v) NaOH/ 12 % (w/v) urea solution and incubated at -$20\,^oC$ for 6 hours. Afterwards, the solution was thawed and neutralized to pH 7, washed with diH_2O, and dried.
- H_2O heat reflux: 100 mg of shoddy was placed in 10 mL of diH_2O and refluxed at 100^oC for 30 min. After, the shoddy was filtered from solution and dried.

Pretreated shoddy (100 mg) was transferred to a 4-dram (14.8 mL) borosilicate vial with 100uL of Cellulase enzyme (100 FPU mL^{-1}) in 9.90 mL 50 mM Na-Citrate buffer (pH 4.8). The hydrolysis was conducted for 96 hours and 0.5 mL aliquots were taken every 24 hours. To isolate the enzyme from the supernatant, the aliquots were centrifuged using Pall Corporation Nanosep 3k Omega Centrifuge Tubes (5000 g for 1 hour). Following 96 hours, the remaining supernatant was collected, and the shoddy was rinsed with deionized water through a stainless steel 150-micron sieve and dried.

3.6.2 Cutinase Hydrolysis Using Melt-Quench Pretreatment

This specific experiment attempted to rectify the obstacles faced during the initial enzyme cascade experiments. Three 100 mg shoddy samples were weighed and placed in their respective aluminum tin tray. One experiment placed the trays in a 350 ^{o}C oven for 10 minutes before immediately dunking the heated shoddy into liquid nitrogen (until bubbling subsides). Each of the heat quenched shoddy samples were then placed in 4-dram glass vials; the vials consisted of 9.90 mL 50mM Na-Citrate buffer (pH: 8.0) and 100 uL of Cutinase enzyme (113 U mL^{-1}). These vials were positioned in a 75 ^{o}C sand bath.Similar to previous hydrolyses, this experiment ran for 96 hours and 500 uL aliquots were collected every 24-hour mark. After the completion of the 96 hours, the aliquots were centrifuged using Pall Corporation Nanosep 3k Omega Centrifuge Tubes (5000g for 1 hour). The remaining shoddy was rinsed with diH2O through a stainless steel 150-micron sieve. One procedure then oven dried the shoddy at 350 ^{o}C for 25 minutes and weighed the final mass of the shoddy.

3.6.3 Enzymatic Cascade

The enzymatic cascade ran a total of 192 hours since it was broken down into two 96-hour hydrolysis steps. To a 4-dram vial, 100.0 mg of shredded shoddy sample # 12, 9.90 mL 50 mM Na-Citrate buffer (pH: 4.8) and 100 uL of Cellulase enzyme (100 FPU mL^{-1}) were added. After a 96-hour hydrolysis (conducted at 50 ^{o}C), each supernatant was collected for later analysis. One experiment washed the remaining shoddy with diH2O, dried at 350^{o}C for 25 minutes, and weighed the resulting mass.

In the same dram vials, each hydrolyzed shoddy sample was heat quenched (i.e., placed in the 350 ^{o}C oven for 10 minutes and placed in liquid nitrogen). One experiment placed into each shoddy containing dram vial 9.90 mL 50mM Na-Citrate buffer (pH: 8) and 100 uL of Cutinase enzyme (113 U mL^{-1}). Placed in a 65 ^{o}C sand bath, this experiment was conducted for 96 – experiment hours. Throughout the hydrolysis, 200 uL aliquots were taken out every 24 hours. To remove the enzyme from the supernatant, these aliquots were centrifuged using Pall Corporation Nanosep 3k Omega Centrifuge Tubes (5000g for 1 hour). Once the experiment concluded, the remaining shoddy sample was washed, dried, and the weight was recorded.

3.7 Analysis of Enzymatic Hydrolysis Experiments

Analysis of the hydrolysis experiments including the mass change between the shoddy samples' initial mass before pretreatment, mass before being treated with enzyme, and final mass after enzymatic hydrolysis were measured. Glucose liberation over the 96-hour experiment was analyzed using an Amplex Red Glucose/Glucose Oxidase Kit sourced from Thermo-Fisher. Terephthalic acid liberation was quantified using UV-VIS spectroscopy and measuring the absorbance of reaction supernatants at 540 nm. Shoddy composition before and after hydrolysis was probed by FTIR-ATR analysis to observe the disappearance of characteristic functional groups (PET carboxylic acid, C=O, 1760 cm^{-1} and Glucose hydroxyl, -O-H, 1080 cm^{-1}).

3.7.1 Glucose Assay

A glucose assay was performed using the Amplex Red Glucose/Glucose Oxidase Kit sourced from Thermo-Fisher. This kit works by utilizing the glucose oxidase which reacts with the free glucose monomers to produce gluconolactone and hydrogen peroxide. The resulting hydrogen peroxide then reacts with the Amplex Red Reagent in a 1:1 stoichiometry to generate resorufin; which can be quantified due to its fluorescence. Figure 1 further depicts this mechanism. The protocol provided by Thermo-Fisher was used to perform the assay. First a glucose standard curve was created (i.e., 0 uM to 500 uM) from a 400 mM glucose stock solution; due to the addition of a reaction mixture, all of these concentrations were two-fold lower (i.e., 0 to 250 uM). The experimental aliquots taken for each time point were used and diluted appropriately. Each sample was loaded into the wells (e.g., 50 uL) and reacted with 50 uL of the prepared working solution of the Amplex Red reagent. After incubating at room temperature for 30 minutes, the absorbance values were obtained at 560 nm.

4. Appendix

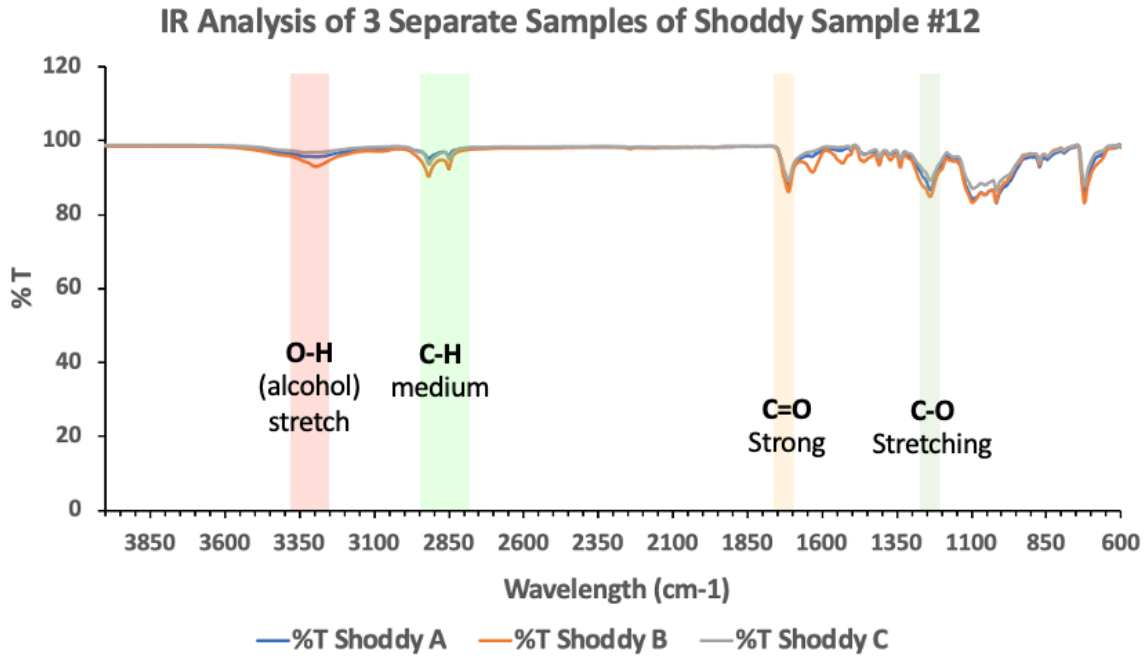

Figure A-1. FTIR-ATR spectra of shoddy sample # 12 (n=3)). Approximately, 1 mg of shoddy sample was collected at random from the total amount of shredded shoddy sample #12. Once collected these samples were placed in a desiccator prior to the FTIR-ATR analysis to eliminate possible moisture exposure in the lab.

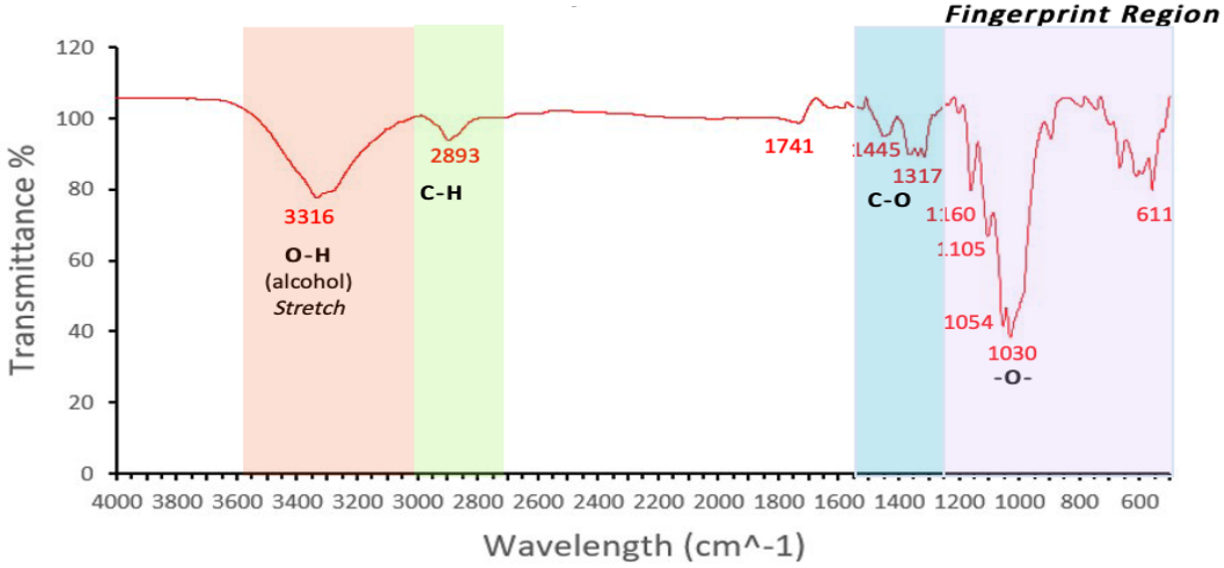

Figure A-2. FTIR-ATR spectra of pure cotton pad.

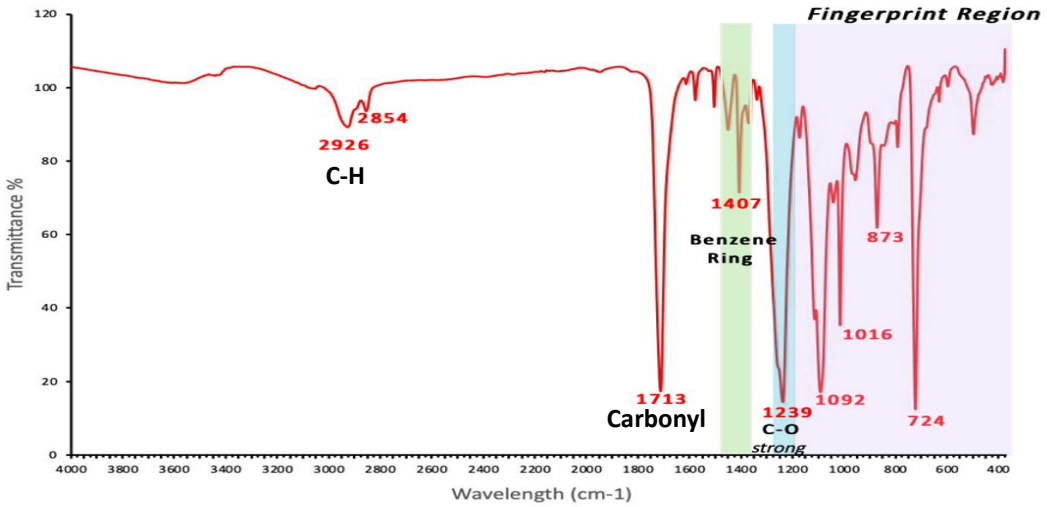

Figure A-3. FTIR-ATR spectra of pure cotton pad.

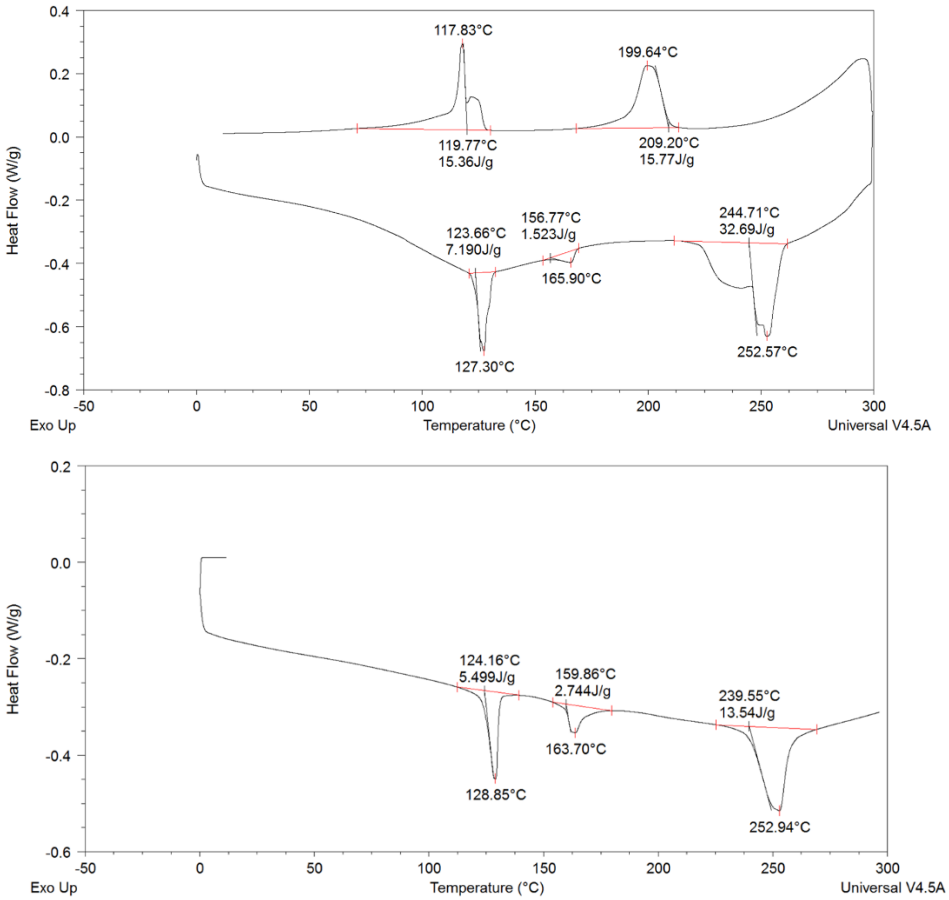

Figure A-4. DSC scans of shoddy sample # 12. The top image is the first heat-cool cycle whereas the bottom image is the second heating run. No cooling cycle was conducted on the second run.

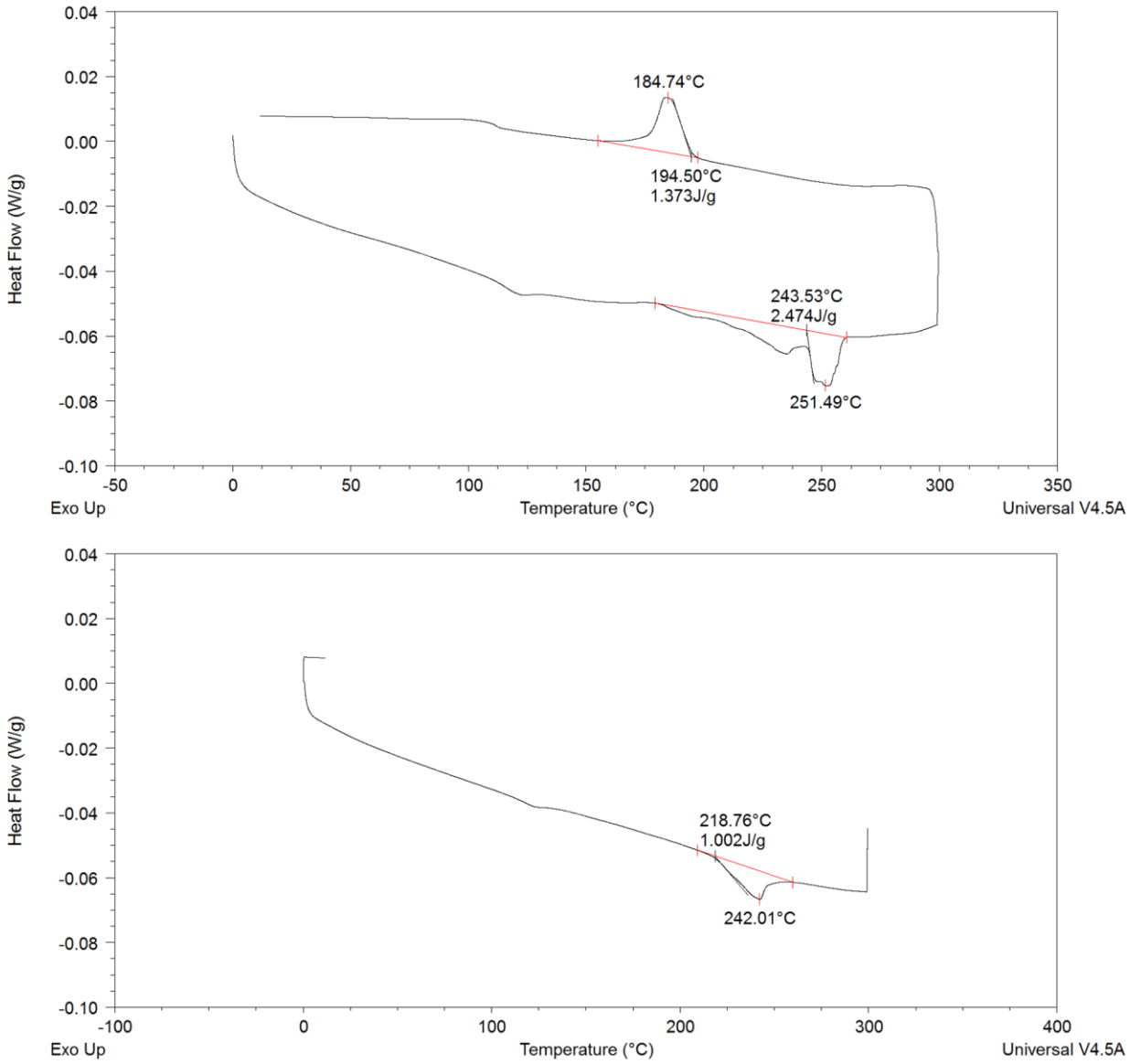

Figure A-5. DSC scans of shoddy sample # 12 post melt-quench protocol. The top image is the first heat-cool cycle whereas the bottom image is the second heating run. No cooling cycle was conducted on the second run.

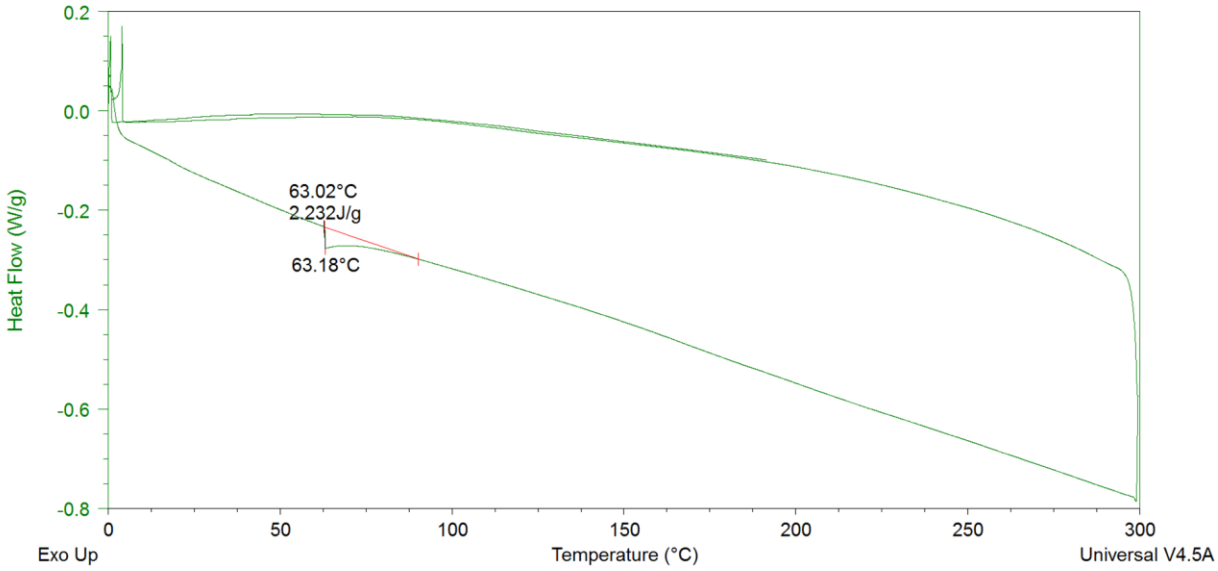

Figure A-5. DSC scan of pure cellulose pad.

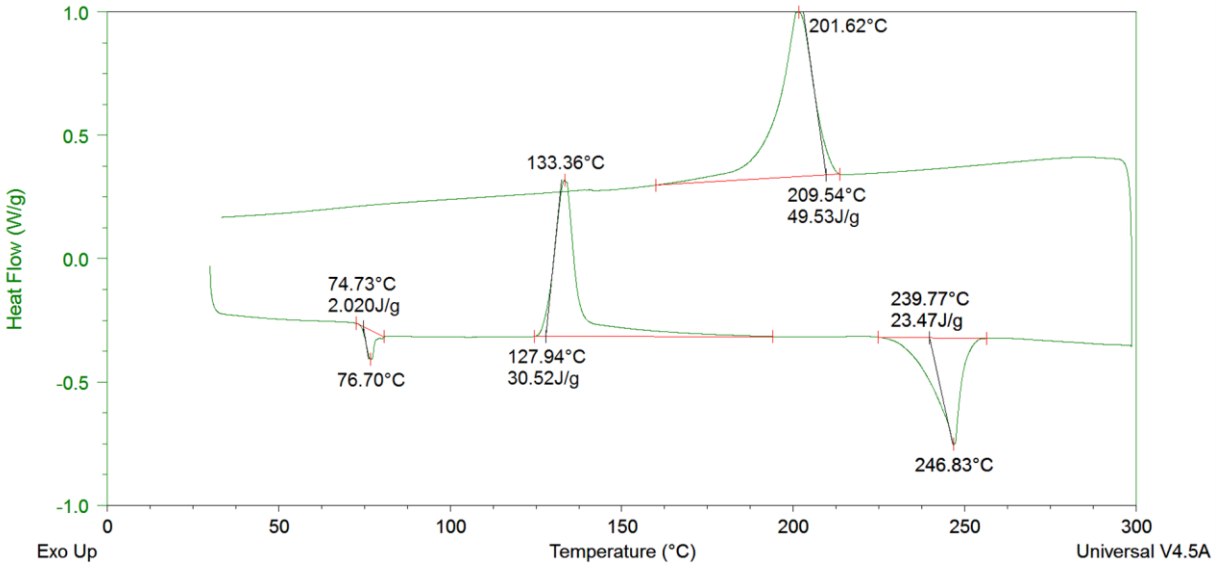

Figure A-6. DSC scan of pure PET.