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Enzymatic Degradation of Fiber-Reinforced PLA Composite Material

Eldor Urinov [1,*], Stefan Hanstein [2] and Anke Weidenkaff [2]

[1] Material Science Department, Lichtwiese Campus, Technical University of Darmstadt, 64289 Darmstadt, Germany
[2] Bioeconomy Department, Fraunhofer IWKS, Brentanostr. 2A, 63755 Alzenau, Bayern, Germany
* Correspondence: eldor.urinov@stud.tu-darmstadt.de

Abstract: Application of thermoplastic fiber-reinforced lightweight composite materials provides a wide range of advantages that are of particular importance for the mobility sector. UD tapes composed of unidirectionally (UD) oriented inorganic fibers embedded in a thermoplastic matrix represent light-weight materials with high tensile strength. This publication addresses recycling aspects of novel UD tape made of a combination of basalt fibers and different PLA (polylactic acid) formulations. The kinetics of enzyme-based separation of polymer from the fiber were investigated. Different types of UD tapes with a thickness of 270–290 µm reinforced with basalt fiber weight ratios ranging between 51 and 63% were incubated at 37 °C in buffer solution (pH 7.4) containing proteinase K. The influence of enzyme concentration, tape weight per incubation tube, proteinase K activators, and tape types on the rate of enzymatic decomposition was investigated. Enzyme activity was measured by analyzing lactate concentration with lactate dehydrogenase and by measuring weight loss of the composite material. The rate of lactate release increased in the first 30 min of incubation and remained stable for at least 90 min. Weight loss of 4% within 4 h was achieved for a tape with 56% (w/w) fiber content. For a sample with a surface area of 3 cm^2 in a buffer volume of 10 mL, the rate of lactate release as a function of enzyme concentration reached saturation at 300 µg enzyme/mL. With this enzyme concentration, the rate of lactate release increased in a linear manner for tape surface areas between 1 and 5 cm^2. Four tapes with different PLA types were treated with the enzyme for 17 h. Weight loss ranged between 7 and 24%. Urea at a concentration of 0.5% (w/v) increased lactate release by a factor of 9. Pretreatment of tapes in alkaline medium before enzymatic degradation increased weight loss to 14% compared to 5% without pretreatment. It is concluded that enzymatic PLA hydrolysis from UD tapes is a promising technology for the release of basalt fibers after alkaline pretreatment or for the final cleaning of basalt fibers.

Keywords: UD-tape; enzymatic degradation; hydrolysis; proteinase K; lipase; PLA; basalt fiber

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1. Introduction

Fiber-reinforced composite materials are extensively used in various applications in which high mechanical strength and stiffness are required at a low weight. Yet, the task of recycling these materials has not been solved at an industrial or commercial level. For Glass Fiber Reinforced Polymers (GFRP), the task has been on the agenda for 30 years [1]. Although recycling methods have been developed, the market has not adopted recycled fibers for several reasons. One important reason is the "stigma" of poor quality [1]. Here we demonstrate the recycling method of the fiber by gently hydrolyzing the polymer matrix of the composite material. This composite material is composed of basalt fiber and polylactic acid (PLA), which has high mechanical performance at a noticeably low weight. In the first place, it is a thermoplastic composite material with a polyester matrix, which can be removed at ambient temperature by hydrolysis of the ester bond [2]. This circumvents the difficulties with the removal of thermoset matrices, which are common in industrial

GFRP products. Secondly, compared to glass fibers, basalt fibers can be produced at lower temperatures but show higher corrosion resistance [3].

The composite material was used as UD-tape for this project. Unidirectional endless basalt fibers containing tapes were produced by Fraunhofer IMWS through the melt impregnation process using tailored PLA formulations from Fraunhofer IAP and Fraunhofer ICT. Polylactic acid (PLA) is one of the well-known aliphatic polyesters that are biodegradable and are produced from agricultural resources such as corn [4] and through ring opening polymerization (ROP) of the lactides [5]. Because of its good mechanical performance and its biodegradability, PLA is widely applied in medical and packaging applications. In order to improve its mechanical properties, various fiber-reinforced PLA composites have been developed, including types containing carbon and polyamide fibers [6]. The degradation of PLA happens through different mechanisms; for instance, microbial, enzymatic, oxidative, hydrolytic, thermal, chemical, and photodegradative mechanisms. Enzymatic and microbial degradation mechanisms are of particular interest since these mechanisms provide a condition which enables the PLA to degrade down to monomer units [7–9]. The mechanism of the enzymatic degradation of PLA substrates is based on the adsorption of the enzyme molecules onto the surface of the PLA and then catalyzing the hydrolysis of its polymeric structure. The hydrolysis of the PLA molecules occurs by cleavage of the ester bonds in the polymer chain [10] and, as a result, produces lactic acid oligomers and monomers [11].

When the UD tapes of this investigation were incubated in 4 M NaOH, weight loss reached the nominal PLA content of the tape within a few hours (Urinov, unpublished results). When the treatment was continued, weight loss exceeded PLA content, indicating the risk of fiber etching with alkali. The enzymatic degradation rate of PLA highly depends on the physical and chemical properties of PLA, the ambient medium, and the enzyme. Surface roughness and PLA density at the tape surface may play an important role in enzyme access to the PLA substrate. Regarding the molecular weight range of the PLA, esterase enzymes like, for example, *Rhizopus delemar* lipase, hog pancreatic lipase, and carboxylic esterase are well suited to induce the hydrolysis reaction of low molecular weight PLA, whereas the degradation of high molecular weight PLA has been reported for other hydrolytic enzymes like proteinase K [12]. Nóra Hegyesi et al. have compared lipase from *Candida rugosa* (CRL) and proteinase K from *Tritirachium album* as a catalyst for the degradation of PLA/cellulose nanocrystal composite material. The weight loss of neat PLA in the presence of CRL (pH 7.2) remained small at 1.5% within 60 days. For proteinase K, weight loss was 13–14% within eight hours. Since the release of lactic acid into the reaction medium influences the pH, the degradation rate of PLA slows down. When the enzyme solution is refreshed, the degradation rate recovers [13].

Our investigation has the objective of evaluating the potential of enzymatic PLA hydrolysis for the recovery of commercial basalt fibers (about 53% SiO2, 16% Al_2O_3, 10% Fe_2O_3, 9% CaO, and 5% MgO) from UD tapes. Due to the interaction between a fiber/nanoparticle surface and a polymer matrix [14–17], the cohesion between fiber and polymer molecules has an important influence on the degradation of the polymer matrix. The effect of inserted nanoparticles (TiO_2 and ZnO) on the degradation of PLA with proteinase K was studied by Antonella, Marra et al. The increase of TiO_2 content in the PLA matrix increased the degradation rate of PLA compared to neat PLA. In contrast to TiO_2 nanoparticles, the existence of ZnO nanoparticles decreased the weight loss of the PLA compared to the neat PLA [18]. We included a degradation experiment with neat PLA.

2. Materials and Methods

UD-tapes were supplied by Fraunhofer IMWS in Halle. The thermoplastic matrix was reinforced with basalt fibers in a melt-impregnation process. Unless otherwise stated, Ingeo 3251D (NatureWorks LLC) was used. On a mass basis, fiber content was 56%. Improved PLA formulations were provided by Fraunhofer ICT (Pfinztal) and Fraunhofer IAP (Potsdam). The fiber content of these composites was in the range of 51–63%. A lyophilized

proteinase K, ≥30 mAnson U/mg, isolated from *Tritirachium album* (haemoglobin, pH 7.5, 25 °C) was supplied by Carl Roth GmbH + Co. KG, Karlsruhe (Art. No. 7528.1). The enzyme was dissolved in 5 mL of ultrapure water and stored in aliquots at −20 °C. The protein concentration was 20 mg/mL. L-lactate was measured with enzymatic test kits from R-Biopharm, Darmstadt (Art. No. 10139084035). All the kit components, except bottle 2, were used as supplied. The content of bottle 2 was dissolved in 6 mL of distilled water. Sodium azide was from Sigma-Aldrich (purified 99.5%). All other reagents were purchased from Sigma-Aldrich at analytical grade.

2.1. Enzymatic Degradation with Proteinase K

Tape pieces were placed in plastic tubes containing 10 mL of Tris-HCl buffer (pH 8.6), proteinase K, 2 mg of sodium azide, 5 mM $CaCl_2$ (preventing autohydrolysis), and distilled water. Unless otherwise stated, the enzyme activity in the incubation medium was adjusted to 9.13 mAnson-U/mL, corresponding to 0.3 mg proteinase K/mL, and the tape surface area was 3 cm^2. The tape thickness was between 0.27 and 0.29 mm. The reaction was carried out in an overhead shaker (70 rpm) at 37 °C. After the specified reaction time, the sample was taken out of the solution and washed three times, filtered, and dried in a drying oven at 40 °C for 1 h. For experiments with successive sample weight determinations (Figures 3 and 5), the buffer/enzyme solution was replaced before the next incubation interval, in order to keep enzyme activity and pH stable.

2.2. Lactic acid Concentration Measurement

Degradation assay samples were heated in a water bath at 100 °C for 10 min to inactivate proteinase K before being frozen. The measurement of the L-lactate concentration is performed by UV-photometry (Hach, DR3900) of NADH according to the protocol of the test kit supplier. The measurement principle is based on the following two-step enzymatic reaction:

$$\boldsymbol{L-lactate} \;+\; \boldsymbol{NAD^+} \overset{L-LDH}{\leftrightarrow} \boldsymbol{pyruvate} + \boldsymbol{NADH} \;+\; \boldsymbol{H^+}$$

$$\boldsymbol{Pyruvate} \;+\; \boldsymbol{L-glutamate} \overset{GPT}{\rightarrow} \boldsymbol{L-alanine} \;+\; \boldsymbol{2-oxoglutarate}$$

In the first reaction, the amount of NADH that is produced by L-lactate dehydrogenase (L-LDH) is stoichiometric to the amount of L-lactate. Complete conversion of L-lactate is accomplished by preventing the backward reaction since pyruvate is converted in the subsequent reaction catalyzed by glutamate-pyruvate transaminase (GPT) in the presence of L-glutamate. The increase in NADH is determined by measuring its UV absorbance at 340 nm. The procedure was performed according to the protocol of the supplier. NAD^+, L-glutamate, GPT, distilled water, and sample solution were thoroughly mixed in assay tubes. A blank assay was prepared in parallel with water instead of the sample, in order to correct for the increase in absorbance caused by the addition of L-LDH (see below). After equilibration for 5 min, absorbance A1 was measured. L-LDH was added. After 30 min, absorbance A2 was recorded. The concentration of L-lactate was calculated according to Equation (1):

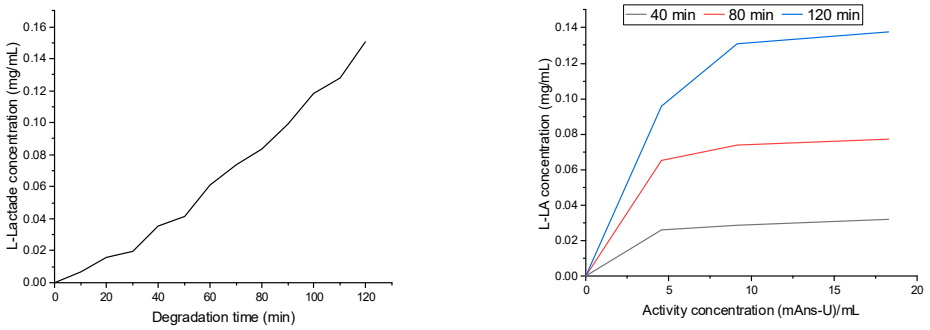
$$c_{L-LA} = \frac{V \cdot M_w}{\varepsilon \cdot d \cdot v \cdot 1000} \cdot \Delta A^* \; [g/l] \tag{1}$$

V = final volume [mL]
v = sample volume [mL]
M_w = molecular weight of the substance to be assayed [g/mol]
d = light path [cm]
ε = extinction coefficient of NADH at: 340 nm = 6.3 [l × $mmol^{-1}$ × cm^{-1}]
$\Delta A^* = \Delta A_{sample} - \Delta A_{blank}$
$\Delta A_{sample} = A_2 - A_1$
$\Delta A_{blank} = A_2 - A_1$

3. Results and Discussion

3.1. Enzymatic Degradation of PLA-UD Tapes

PLA composite material was degraded in the presence of 9.13 (mAns-U)/mL proteinase K at 37 °C. Figure 1a shows the concentration (mg/mL) of produced lactic acid as a function of degradation time. After two hours, the concentration of lactate was approximately 150 µg/mL, corresponding to 1.5 mg of lactic acid in the total liquid volume of 10 mL. The measured weight loss of the same sample was 2 mg, indicating that a major part of PLA was converted to monomers. This was confirmed in an experiment with an incubation time of 12 h, in which the weight loss was 25 mg, while 15.9 mg of lactate was released.



(**a**) (**b**)

Figure 1. Lactate concentration during enzymatic PLA degradation (activity concentration of the enzyme is 9.13 (mAns-U)/mL) as a function of time (**a**) and enzyme activity concentration (**b**).

In order to study the influence of enzyme concentration on the degradation rate, the produced L-lactate concentration was measured at three different enzyme activities: 4.57 (mAns-U)/mL, 9.13 (mAns-U)/mL, and 18.26 (mAns-U)/mL (Figure 1b). At the lowest enzyme activity concentration, the concentration of L-lactate was 25 µg/mL, 65 µg/mL, and 96 µg/mL after 40, 80, and 120 min, respectively. Within the first 40 min, a further increase in the enzyme concentration did not cause a significant change in L-lactic acid release. However, when the reaction time was extended beyond 40 min, the doubling of enzyme concentration caused a noticeable increase in L-lactate release, particularly after 120 min. Further increases in enzyme concentration had no effect on lactate release, even after 120 min.

With the intermediate enzyme concentration equivalent to 9.13 (mAnson-U)/mL, the rate of L-lactate release was proportional to the size of the UD tape (Figure 2), showing that the amount of PLA released per area unit of UD tape remained constant up to the largest surface area tested (5 cm^2). It can be concluded that Figures 1b and 2 consistently show that the intermediate enzyme concentration is not a limiting factor for the rate of PLA degradation.

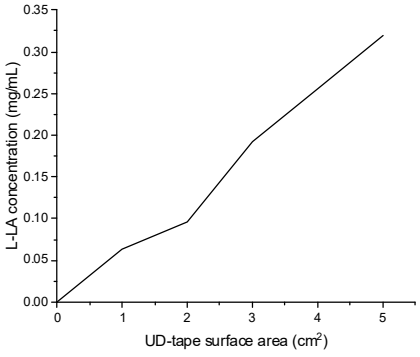


Figure 2. Effect of sample size on L-lactic acid release. Incubation time was 120 min.

3.2. Enhancement of Enzymatic Degradation by Alkali Pretreatment and Urea

In order to increase the degradation rate, UD tape was incubated in a 1 M NaOH solution at 40 °C for 10 min. After this treatment, the sample was washed three times with distilled water and transferred to a proteinase K solution. Figure 3 shows the noticeable increase in weight loss for a tape that has been pretreated with alkali. Without alkaline pretreatment, weight loss through proteinase K activity was about 5% after four hours. This value increased to 14% for the pretreated sample. After subtracting the initial weight loss caused by alkali of about 4%, it is evident that the rate of enzymatic PLA degradation is higher for a PLA surface which has been pretreated with alkali. Underlying mechanisms could be a larger surface area and weaker interactions between PLA and basalt fibers. Furthermore, Xu et al. have shown that alkaline treatment of PLA brushes changes the surface chemistry, reducing the contact angle of aqueous solutions and increasing the hydrophilicity, which is likely to favor binding of hydrophilic enzymes [19].

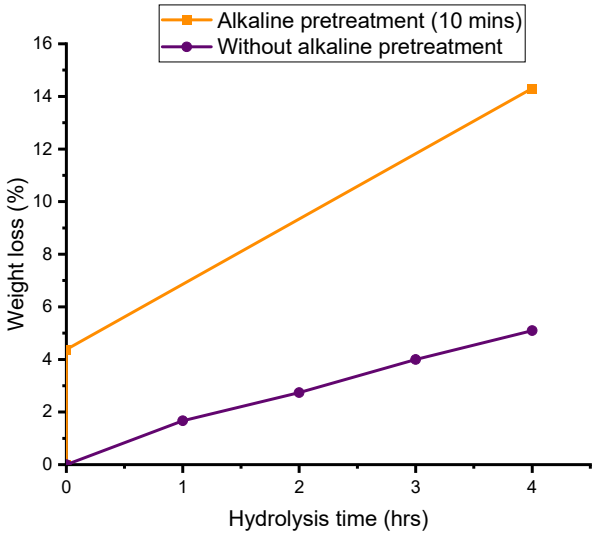


Figure 3. Alkaline pretreatment effect on PLA degradation.

Urea strongly increased the activity of proteinase K. A representative experiment is shown in Figure 4. At a urea concentration of 3% (w/v), PLA degradation increased by a factor of 5.

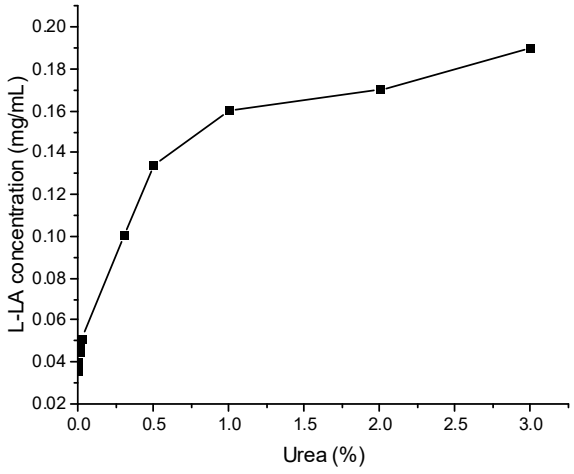


Figure 4. Effect of urea on the enzymatic degradation of PLA. The hydrolysis was carried out for 30 min.

3.3. Effect of Fiber Content and PLA Type on the Degradation of PLA

The influence of the fiber content on the PLA film degradation was compared by degrading samples with fibers (UD tape) and without fibers with proteinase K. The pure PLA film was prepared under lab conditions from the commercial PLA grade, which was also used for the UD tape. Figure 5 shows weight loss of the pure PLA film and fiber reinforced PLA film after contact with proteinase K solution. Weight loss in the first hour was slightly higher for the pure PLA sample compared to the composite PLA. The weight loss rate increased by a factor of 2.5 for pure PLA during the second hour, whereas it remained stable for the fiber reinforced PLA. The weight loss measurement after 4 h confirmed that the presence of fibers decreased the degradation rate.

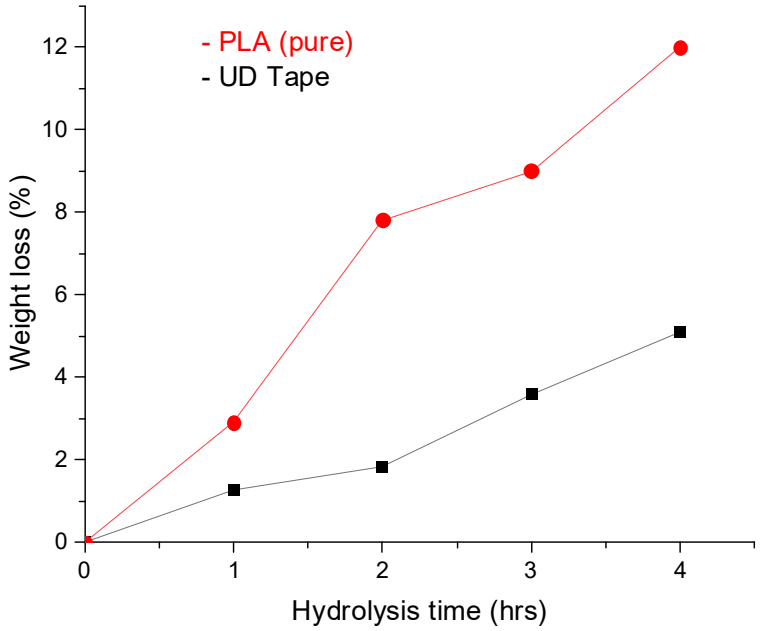


Figure 5. Weight loss (wt.%) of pure PLA and fiber reinforced PLA (fiber 56%). The weight loss of the reinforced PLA is normalized to the overall mass of the UD tape.

Figure 6 illustrates the range of degradation kinetics for different UD tapes. The smallest weight loss for the tape with the highest fiber mass ratio is in line with the previous finding that the fiber content reduces the rate of PLA hydrolysis (Figure 5). However, another important factor is the type of PLA, which was different for UD50-292, UD50-293, and UD50-294. The profound influence of PLA type and formulation is evident by comparing the 51% and 52% fiber content in Figure 6. They were produced by different Fraunhofer Institutes. UD tapes with 52% and 55%, on the one hand, and 51% and 63%, on the other hand, were provided by the same institutes. All PLA formulations are subject to non-disclosure agreements. However, it was reported in an industry report that the UD tape with 63% fiber content achieved the highest tensile strength of 690 MPa [20]. According to Ku.H et al. [21], besides fiber content, the interfacial matrix-fiber bonding is the key element to increasing the tensile properties of the fiber reinforced polymer composite. The tensile strength increases up to a certain level by increasing the fiber content and then drops. Figure 6 illustrates that for UD tapes with superior mechanical properties based on basalt fibers embedded in PLA, enzymatic breakdown is still feasible.

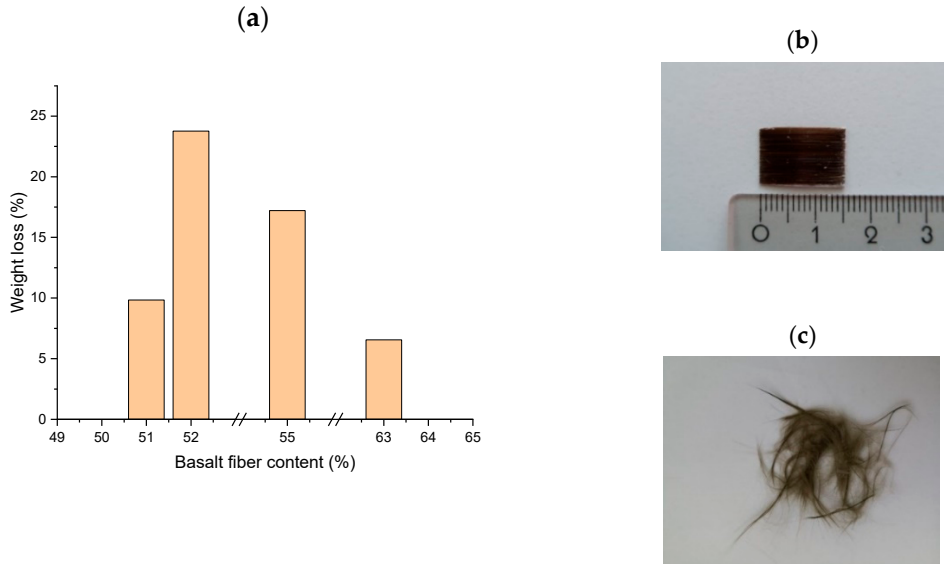


Figure 6. Weight loss for UD tapes with different fiber content (**a**) UD50-291(fiber 63%), UD50-292 (fiber 51%), UD50-293 (fiber 55%), and UD50-294 (fiber 52%). The weight loss of the reinforced PLA is normalized to the overall mass of the UD tape. The degradation time is 17 h for all samples. UD50-294 before (**b**) and after (**c**) degradation.

3.4. Estimates for Industrial-Scale Hydrolysis

The saturation kinetics of Figure 1b shows that with 1 mg proteinase K per cm^2 tape surface (both sides), the rate of hydrolysis is close to maximum. Experiments were performed with tape samples with a total surface area of 3 cm^2 in a buffer volume of 10 mL (tape surface 0.3 cm^2 per mL). The buffer solution was charged with 0.3 mg proteinase K per mL (enzyme concentration of 300 ppm), which is equivalent to 1 mg enzyme per cm^2. The enzyme charge is sufficient to maintain near-maximum hydrolysis rates at higher tape charges of 50 mm^2/mL, corresponding to an enzyme charge of 0.6 mg/cm^2 (Figure 2). Due to the fact that the enzyme was used at a temperature of 37 °C, which is below its optimum temperature, considerably higher hydrolysis rates can be obtained. The optimum temperature of proteinase K was around 65 °C for caseinolytic activity [22] and 56 °C for DNA recovery from proteins [23]. Thermal stability is increased in the presence of calcium ions [24]. Kenjiro et al. discovered that stabilization with heavy atoms (praseodymium) increases catalytic activity 46-fold at 70 °C using synthetic nitroanilide substrate and 9- and 76-fold at 70 and 80 °C, respectively, by using fluorescein isothiocyanate-labelled casein compared to the native enzyme [25]. Therefore, an industrial-scale process of UD-tape degradation may be operated at around 55 °C with at least twofold speed compared to 37 °C. Alkaline pretreatment has been shown to double the hydrolysis rate (Figure 3). Furthermore, when adding 3% urea, the hydrolysis rate increased by a factor of five (Figure 4). Considering these three accelerating factors, it is reasonable to assume that for the different UD tape types shown in Figure 6, complete PLA hydrolysis can be obtained within less than 17 h.

As an example, for the enzyme amount which is required for recovery of 1 kg of fibers, the specification of UD tape 50-291 (Figure 6) will be used. With a surface areal weight of 24.9 mg/cm^2 (surface of both sides) UD tape and a fiber content of 63 wt.%, a fiber weight of 15.7 mg/cm^2 UD tape surface area can be calculated. For an enzyme charge of 0.6 mg/cm^2 tape surface, 1 kg of enzyme is sufficient to recover 26 kg of fibers. With prices of industrial enzymes in the order of EUR 10–20, the resulting enzyme costs per kg of fiber are below EUR 1.

A large potential to reduce the enzyme amount arises from a decrease in the volume of enzyme solution which is used per m^2 of tape surface. The present value is 33.3 L/m^2 (3.33 mL/cm^2). Assuming that (a) the enzyme would have a density of one, (b) the complete

enzyme charge of 6 g/m^2 (0.6 mg/cm^2, see above) would adsorb to the surface, and (c) surface roughness would increase the physical surface by a factor of ten, a layer with a thickness of 600 nm would form, which is far beyond an enzyme monolayer. Thus, it is conceivable to suggest a reduction of the enzyme solution volume per m^2 tape surface to 1/10 of the used ratio, which would lower the enzyme demand accordingly. The weight concentration of UD tape in the enzyme solution would then be 7.5%.

4. Conclusions

For UD tapes based on PLA and basalt fibers, enzymatic PLA hydrolysis with proteinase K can be employed for fiber recovery within 17 h. The hydrolysis rate is increased by alkaline pretreatment and urea addition in the enzyme buffer. The presence of the fiber molecules decreased the rate of PLA weight loss. For industrial recovery of fibers, enzyme costs per kg of fibers are in the range of EUR 0.1–1.

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