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Effective Pretreatment Routes of Polyethylene Terephthalate Fabric for Digital Inkjet Printing of Enzyme

Tuser Biswas, Junchun Yu, and Vincent Nierstrasz*

Enzymes immobilized on synthetic polyethylene terephthalate (PET) textile surface by resource-efficient inkjet printing technology can promote developments for various novel applications. Synthetic fabrics often require adequate pretreatments to facilitate such printing process. This work discusses PET–woven fabric pretreatment routes to improve wettability by alkaline, enzymatic, and plasma processes for effective printing of lysozyme using an industrial piezoelectric printhead. Results indicate that all pretreated samples contain similar amount of enzymes upon printing. Plasma treated fabrics show relatively more hydrophilic surface characteristics, better protein binding stability, and lower retained activity. Alkali and cutinase-treated samples possess relatively higher activity due to greater amount of enzyme desorption to substrate solution. Depending on respective enzyme-binding stability, combination of a well pretreated surface and inkjet as preferential placement technology, the approach of this study can be used as a facile enzyme immobilization method for suitable applications, for example, controlled-release and bio-sensing.

1. Introduction

Enzymes used in solution form often imply challenges on possible recovery, effluent handling, downstream processing, and purification cost. Immobilized enzyme on solid support can resolve such concerns by ease of separation from substrate solution. Additionally, it can minimize product contamination by activity residues. Immobilized enzymes can be effectively reused in continuous processes, with considerable savings in quantity, labor, and overhead cost.[1] Inert polymeric fibrous materials are ideal supports for such immobilization, providing higher loading capacity of biomolecules and larger surface area for interaction.[2] A major number of studies used enzymes during pre-processing in the form of beads, pellets, otherwise during post-processing, for example, surface cleaning and effluent treatment.[2,3] A few studies used flat film and paper like supports, however, use of non-flat textile like surfaces for immobilization is very limited.[4] Textile fabrics can be a more suitable support for enzyme immobilization due to their inherent advantages of being strong, flexible, and lightweight, along with providing low pressure drop in chemical processes. Thus, they show potential to be used in a range of advanced applications, for example, controlled release, drug delivery, bacterial inhibition, and biosensing.[5] Such advanced application often requires highly controlled, precise, contactless, and customizable production method such as digital inkjet printing.[6] Compared to conventional production methods, for example, coating, finishing and screen printing, resource efficient inkjet technology minimizes use of water, energy, chemical and wastes of valuable functional materials, for example, enzymes.[7]

Drop-on-demand (DOD) inkjet technology has been successfully used for printing of enzymes on textiles for various applications, compared to the continuous system.[8] Among two ejection mechanisms of DOD printheads, that is, thermal and piezoelectric, the latter one is preferred due to less possible influence on the protein structure of enzymes and resulting activity.[9,10] Along with printhead mechanics, inkjet printing of enzyme on textiles comes with challenges on ink recipe optimization for a specific enzyme-printhead combination and ensuring proper fabric–enzyme interaction.[11] Optimization strategy of ink-containing enzyme for rheological, ionic, and printhead parameters has been demonstrated in our previous work.[12] In this work, fabric surface characteristics necessary for efficient inkjet printing and retention of active enzymes are studied.

Polyethylene terephthalate (PET) fiber based synthetic products are being used in applications ranging from apparel and home furnishing to medical textiles with rising market trend for more advanced applications.[13] This inert fiber offers superior physiochemical and mechanical properties. Nevertheless, such fibers retain challenges for printing because of their hydrophobic surface caused by lack of polar groups. Additionally, synthetic fiber surfaces may induce greater hydrophobic interaction with enzymes, for example, lysozyme to cause confirmation changes and denaturation.[14] Due to this fact, studies regarding immobilization of this enzyme on synthetic fiber

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surface are limited, especially using a printing technology.[15] Effective printing would require to improve wettability of PET surface through a suitable pretreatment process that can facilitate proper binding of enzymes and then to retain satisfactory activity. Wettability of PET can be improved to various degrees through well-studied approaches like alkaline, enzymatic, and plasma based pretreatments, each with certain effects on resulting physiochemical and mechanical properties. However, effect of these treatments for inkjet printing of enzymes are yet to be studied. Alkaline (sodium hydroxide) based PET treatment is the cheapest and most conventional process in industries that can greatly improve wettability, though with expense of high chemical, water and energy consumption, along with probable damage to mechanical and aesthetic properties under certain conditions.[16–19] Enzymatic (cutinase) based treatments are somewhat costlier (depending on purchase volume), but able to improve wettability significantly with lower impact on resources and physio-mechanical properties of PET fiber.[16,17,20–22] Atmospheric plasma can bring about similar improvement as alkaline method with less effect on the bulk properties and resource consumptions, though it requires comparatively higher initial investment.[22–25] PET surface modification for effective enzyme printing could be achieved by any of these processes after appropriate parameter adjustments to minimize effects on used resources, environment, and mechanical properties of fiber.

Lysozyme is a well-studied antimicrobial enzyme that can be used as a model protein for immobilization studies.[26,27] It has been grafted in modified PET and other polymeric surfaces mostly through covalent binding involving use of strong fixatives, for example, glutaraldehyde.[28–31] Nevertheless, possibilities of simple adsorption of the same on pretreated fiber surfaces without use of additional chemicals are not well explored, especially involving digital print technology. Better understanding of lysozyme activity upon printing on such pretreated surfaces are necessary as well. Therefore, this study aimed to find an optimum PET fabric pretreatment route for inkjet printing of lysozyme with well-retained activity.

2. Results and Discussion

2.1. Pretreatment Effects

Effective inkjet printing of enzymes on hydrophobic PET fabric would largely depend on the improvement of surface wettability. Three PET pretreatment routes, for example, alkaline (NaOH), enzymatic (cutinase), and plasma (atmospheric) were used in this study to increase wettability by adapting their well-established methods in literature.[16,17,22–24] The aim was to find an optimum pretreatment route for improving wettability with minimum compromise of physical–mechanical properties and yet, maintaining satisfactory activity of printed enzyme.

Table 1. Result of different pretreatments on physical–mechanical properties of PET fabric.

	Pretreatments			
	Untreated	Alkaline	Cutinase	Plasma
Water contact angle	109° ± 4°	67° ± 4°	71° ± 5°	43° ± 7°
Weight loss	–	3.9%	0.7%	0.2%
Tensile strength reduction	–	4.3–5.7%	0.6–0.9%	1.4–1.7%

Following the pretreatments, all samples showed improved wettability by significant reduction of contact angle, along with varied amount of weight and strength loss as presented in **Table 1**. Proving to become more hydrophilic, plasma-treated fabrics had higher reduction of water contact angle (WCA) of about 66°, compared to about 40° reduction for alkali and cutinase treated fabrics. Alkali treated fabrics showed highest loss of weight and strength (≈4–5%). Cutinase treated fabrics had low to moderate change on these aspects (<1%). Plasma treated fabrics showed lowest weight loss (0.2%), however significant reduction of tensile strength (≈1.5%). These results are within the range as found by above referred pretreatments studies. Although, the pretreatment methods were adapted to retain similar properties on all samples, it was difficult to achieve the same effect due to their difference in surface modification mechanisms. Alkaline and cutinase treatments cause hydrolytic scission of ester bonds on PET backbone but through different mechanisms, for example, exo- and endo-cleavage and preferably on different regions, that is, crystalline and amorphous, respectively.[17] Both treatments result in degraded products from PET, for example, terephthalic acid and ethylene glycol (mostly by alkali)[16,19] and generation of new hydrophilic functional groups, for example, hydroxyl and carboxyl (mostly by cutinase).[20,32] Along with chemical, morphological changes brought about by the treatments contributed to improve wettability and modified other physical properties. In line with previous stu dies,[16,18,19,21,25] SEM images showed that all the pretreatment processes caused some roughness on PET surfaces, however, to varied extent for different pretreatments (Figure S1, Supporting Information). Larger areas of localized rough patches were seen on alkali and cutinase treated fabrics, compared to smaller but more evenly distributed patches on plasma treated fabrics.

AFM surface topographic analysis **Figure (1)** showed that for all pretreated samples, number of hills (brighter area) and pits (darker area) were increased and resulted in higher roughness depths. Some roughness was observed in the untreated sample that might have resulted due to pre-wash procedure (Figure 1a). Alkali treated fabrics obtained highest surface roughness with largest heights of pits and hills (Figure 1b), similar to observations of previous studies.[16,17,32] Along with improving wettability, creation of such cavities or voids might have resulted in reduction of weight and strength for this treatment.[17,32] Alkaline process has been predominantly observed to increase porosity of PET structure.[16,33] Conversely, cutinase treated samples relied mostly on chemical modification [16,17,21] with lowest heights of single hills and pits, but possibly merged over a larger area and creating a nearly smoothened surface (Figure 1c). Such overall lower induced roughness contributed to a most gentle effect on fabric strength.[16,21] Referring to these variations, a number of studies [16,17,32] have stated a difference in WCA of about 10° between alkali and cutinase treated PET samples. However, we observed similar WCA for two treatments (Table 1) probably due to adapting optimized experimental parameters and using a stable industrial

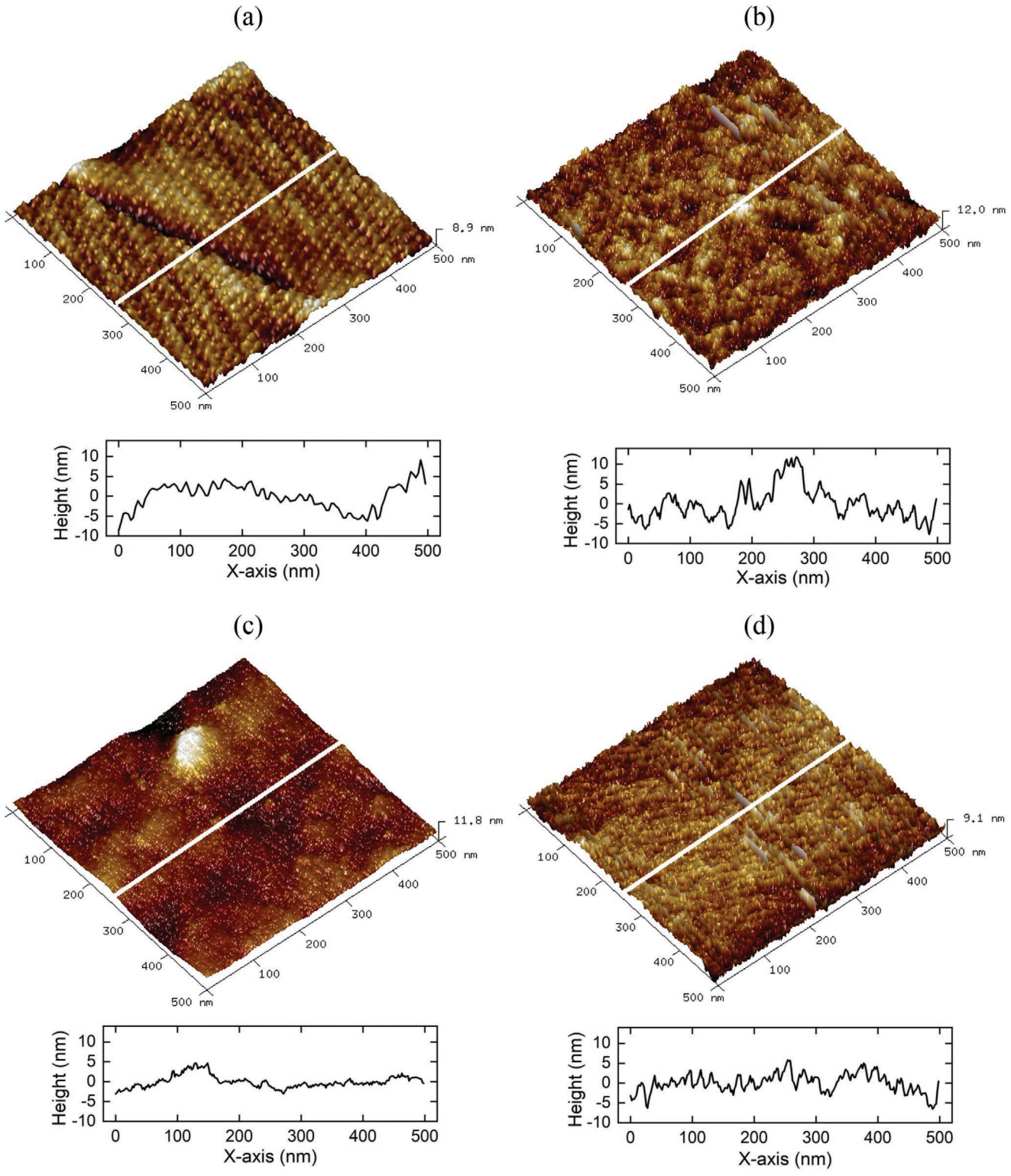

Figure 1. AFM topographic images of untreated a) PET fabric and after pretreatment using b) alkali, c) cutinase, and d) plasma and respective cross-sectional heights along the middle of axis (white line).

cutinase.[21,34] Highest reduction of WCA was observed for plasma treated fabrics owing to both, increased polarity (see results of **Figure 2**a) and creation of ordered scale like structure on PET surface (Figure 1d). This treatment formed uniformly etched surface consisting of higher number of hills and pits with smaller heights than alkaline treated ones. Atmospheric

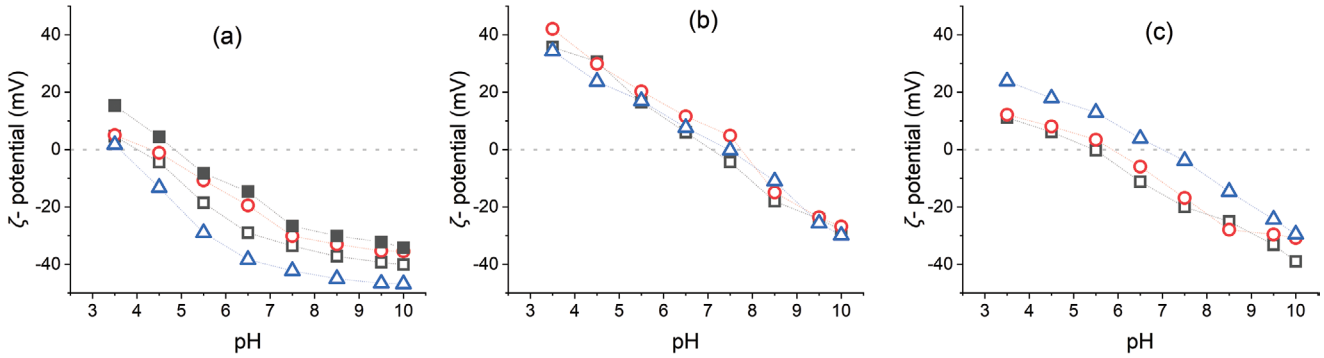

Figure 2. ζ-potential of PET fabric at different stages; a) before printing, b) after lysozyme inkjet printing, and c) after desorption of the printed fabrics in buffer solution. Symbols represent fabrics when un-treated (■) and after treatment with alkali (□), cutinase (○), plasma (Δ).

plasma can cause chain scission of weak surface bonds leading to creation of several polar groups, for example carbonyl, carboxyl, and hydroxyl through oxidation.[25] Additionally, this process increases surface energy and thus, wettability of PET surfaces, without affecting the bulk properties as stated in previous studies.[23,24,35]

2.2. Ink Formulation and Jettability

Ink formulation containing enzyme requires careful consideration of rheology, ionic environment, and printhead parameters to ensure optimum activity of printed enzymes as demonstrated in our previous paper.[12] Considering rheology, viscosity, and surface tension are most necessary to adjust for ensuring proper ejection, drop formation, and spreading of ink on fabric. Glycerol has been demonstrated to be an efficient viscosity modifier for lysozyme solution [36,37] and thus used in our study. However, compared to our previous work,[12] amount of glycerol was reduced in this study considering its humectant nature and thus minimizing post-print drying duration to 1 h (at room temperature). This resulted in ink viscosity of 5.5 mPa.s at 25 °C printing temperature, which is slightly less than printhead manufacturer recommendation range (7–12 mPas). Additionally, to take the advantage of incubation effect of glycerol on lysozyme protein folding,[38] the ink was stored overnight at room temperature before printing.

Surface tension of the ink was controlled by using a non-ionic surfactant to avoid unwanted interaction with lysozyme.[39] Efficient printings require significantly low surface tension of ink than that of water by using a higher amount of surfactant.[8] However, lowering the surface tension may cause enzymes to become susceptible to withstand jetting force and reduce activity due to deformed protein confirmation. Moreover, adding surfactants below the critical micelle concentration (cmc) of ink may promote formation of micelle–enzyme complex to alter activity after printing.[40] Accordingly, our prepared ink had a surface tension of 30 mN m^{-1}, corresponding to a surfactant concentration of just above cmc point. Effectiveness of such rheological modifications were then analyzed by calculating the theoretical jettability of prepared Newtonian ink fluid which can be understood through limiting values of few unitless numbers, that is, Webers number (*We*) and inverse Ohnesorge number (*Z*). These numbers were calculated from density and surface tension of ink, along with velocity and characteristic length of printhead as explained in literature.[6] Our prepared ink had limiting values of numbers ($Z = 3.7$, $We = 8.2$) well within the range for efficient inkjetting process ($1 < Z < 10$; $We > 4$).[41] Obtained *We* and *Z*-values ensured that the ink would overcome the influence of air–fluid interface for drop formation and continuous ejection through printhead nozzles without formation of satellite drops, respectively.

Solubility, activity, and structural stability of enzymes are dependent upon the pH and ionic strength on ink solution.[27,28] Lysozyme was soluble in ink solution over a range of pH 5–10. Lytic activity of the enzyme on its oppositely changed substrate surface as governed by the electrostatic forces acting between them was most prominent at ink pH of ≈7 and 9, similar to its behavior in buffer solution.[26] Along with pH, protein structure stability of an enzyme depends upon ionic strength of solution by regulating the net charge development on residues and difference in Gibbs free energy for destabilizing forces.[42] For the prepared ink, most suitable ionic strength was 0.05 M at pH 7, which is in line with our previous study.[12] At this range, maximum lysozyme coverage on fabric surface could be expected.[15]

An appropriately high concentration of enzyme in the ink solution would ensure a constant rate of catalysis for maximum possible activity of printed fabric. Linear activity range of jetted ink was until the protein concentration of 150 μg mL^{-1} and it increased to about 800 μg mL^{-1} for printed fabrics. Such increase in linear range upon immobilization of enzyme on solid supports has been mentioned in literature [9,43] due to possible restricted and/or slower transportation and diffusion with substrate solution.[44,45] Further increase of concentration had no significant effect on activity due to saturated printed surface.[28] Lysozyme was readily soluble in prepared ink solution and appeared transparent, ensuring less possibility of printhead nozzle clogging. Jetted ink retained 64.4% activity, which is lower than our previous study (≈85%) with same enzyme.[12] This might simply occur due to use of different printheads and corresponding differences in shear stress produced by the jetting force for possible alteration of protein structure. Additionally, comparatively less amount of glycerol used in this study might have compromised the natural protection of lysozyme protein structure [36,37] against jetting force and resulted in such lower retention percentage.

2.3. Enzyme Immobilization

Following printing process, lysozyme was expected to be immobilized over PET surface and inside porous fiber network through simple adsorption.[46] Amount of immobilized enzyme (protein) was determined through XPS measurements of nitrogen (N1s) peaks at the binding energy region of 398–402 eV. Traces of nitrogen (N%) were present on all measured samples (Figure 1), including the untreated PET (<0.1%). Untreated, alkaline and plasma treated samples had N% of lower than the atomic sensitivity factor (<0.477%) for XPS measurement and thus unable to confirm presence of any protein molecule.[47] However, cutinase treated samples showed comparatively higher N%, probably due to insufficient after-washing process which involved use of protease. Although, it might be challenging to remove the protein molecules completely in a gentle enzyme-based pretreatment process,[20,21] the effect can be minimized by prolonged washing or by using a more rigorous soda-based after-wash process.[16]

After printing, all the samples showed significantly higher N% than only pretreated ones (Figure 1). This confirmed presence of lysozyme on printed fabrics. Although, same amount of enzyme was printed on the three sample types, interestingly, plasma treated fabrics showed significantly higher N% than alkali and cutinase treated ones. This could be due to higher adsorption of lysozyme on plasma treated PET fiber surface than toward bulk and XPS measurement being more sensitive to outer surface layers. Additionally, compared to alkaline and cutinase based treatments, plasma process has been stated to induce more oxygen and carboxylic groups on PET fiber which are capable of higher protein adsorption.[35] Functional groups have been mentioned to be present toward outer fiber layer for plasma treated samples and toward deeper surfaces for the other two treatments.[22,48] Therefore, it was not possible to reach any conclusion on the precise amount of immobilized enzymes on printed fabrics from XPS data and required further analysis as discussed in next sections. Presence of the printed ink materials on fiber surface was also observed on SEM images (Figure S1, Supporting Information). Roughness caused by the pretreatment processes were reduced owing to ink glycerol content. Grain-like substances appeared upon printing, which possibly were the buffer salts of ink. Such grains on plasma-treated sample were of smaller sizes and evenly distributed, compared to alkali and cutinase-treated samples. Enzymes were probably mixed with both of these ink contents, however could not be ascertained on SEM images.

2.4. Activity of Printed Enzyme

Microenvironment inside the fabric matrix is expected to ensure greater operational stability of enzymes against denaturation, however, it might cause reduced activity due to unwanted interaction with the immobilized medium, restricted mobility, change of protein confirmation, and inaccessibility of active sites toward substrates.[2] Highest activity found among all the printed samples was only about 2% of jetted-ink activity (when printed and collected on a glass plate). Reason for such activity reduction might be due to change of enzyme–substrate interaction from a macro to micro-environment and corresponding issues of diffusion and transportation. Following our activity assay setup (see method), there are two possible routes for printed enzymes to convert the substrates into products. First, substrates may diffuse through printed fabric pores to reach the catalytic sites depending on surface wettability as discussed previously. Secondly, enzymes may desorb from fabric to substrate solution depending on their adsorption stability and act similarly as enzymes in free solution.[46] Therefore, extent of activity would depend upon in which of the above routes most immobilized enzymes are interacting with substrates. In case of the first route, reduction of activity could result from enzyme disorientation, denaturation, and/or unwanted interaction upon immobilization. For instance, Kubiak–Ossowska [15] has discussed possible orientation of lysozyme active site toward fibers rather than the substrates to hinder activity. It has been observed that lysozyme needs to adopt a flexible conformation for effective adsorption process,[49] especially on irregular surfaces like textiles.[50] Enzyme activity can be affected by such conformational flexibility to induce changes in their secondary protein structure or even, partial unfolding, if not complete denaturation.[51] Another reason for reduced accessibility of substrate to printed lysozyme could be high protein–protein interaction after immobilization on fiber surface.[52] In case of the second route, higher activity could be expected, as it is less prone to immobilization effects. However, desorbed enzymes might not possess similar catalysis ability as of fresh ink, if their protein structure was already compromised during adsorption process. Thus in both cases, a significant reduction in activity is probable. Nevertheless, to ensure better activity retention, it would be necessary to understand the contribution of two routes on resultant activity as discussed in next sections.

Untreated fabric showed inadequate absorption of printed ink to measure and compare the enzymatic activity with pretreated samples. Pretreated fabrics were printed for several passes to observe the effect of immobilized lysozyme concentration on activity (**Figure 3**a). Number of print passes was restricted to five, since ink already started to flow through the back side of fabrics. No detectable activity could be found from the one-pass samples, possibility due to low enzyme concentration. For all type of pretreated samples, activity started to increase gradually until four-passes and then, more drastically upon the fifth print pass. Similar results of significant activity increment after a certain number of print-passes, that is, enzyme concentration has been observed in inkjet printing studies of other enzymes.[29,44] Reason of such activity trend could be that comparatively higher substrate concentration was available for the samples containing lower amount of enzymes. Even after five print-passes fabrics were not saturated by enzymes as activity plateau was not reached, though printed ink started to flow through the back side of fabrics. Therefore, highest possible protein concentration compatible with ink formulations could be used,[28] even after linear range, to achieve higher activity from inkjet printed fabrics, instead of printing for several passes.

Lysozyme activity was highest for alkali treated five print-passes samples, followed by cutinase and plasma treated respectively (Figure 3a). Reason for such activity variation could be related to manner of enzyme deposition, followed

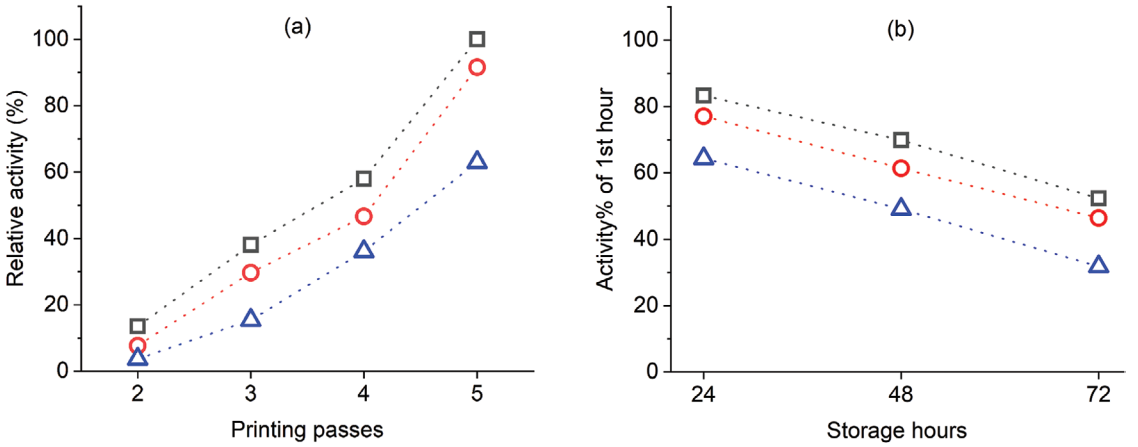

Figure 3. Lytic activity of inkjet printed PET fabrics pretreated with alkali (□), cutinase (o), and plasma (Δ); a) after a number of printing passes (relative to highest observed) and b) after several hours of storage.

by transportation inside fabric structure, and finally, stability after adsorption. Upon inkjet printing, enzymes in pico-liter range were primarily deposited on specific locations of fabric surface. Roughness pattern of such a location could influence further mobility of printed enzymes. Alkali and cutinase-treated samples having larger heights of single or merged hills and pits (Figure 1b,c), could allow deposition of several enzyme molecules at same location and greater space for movement. Comparatively, plasma treated samples having uniform surface depth similar to single lysozyme particle size (3–5 nm), would allow less number of enzymes per site and more restricted mobility. Alkali and cutinase treated samples possessing similar wettability that is significantly lower than plasma treated (Table 1), would allow comparatively limited transportation of enzyme molecules. This could cause some enzymes to stay on outer surface layer with greater possibility to act on substrates. Additionally, activity can depend on the ability of printed enzymes to remain adsorbed on fabric surface brought up by the pretreatments. In case of alkaline and cutinase treated, such adsorption is mainly governed by morphological modifications and induced carboxylic groups.[53] Most stable adsorption could be expected on plasma treated surface due to possible electrostatic interaction as favored by lysozyme,[54] along with morphological changes. However, a strong adsorption would require more flexibility of protein structure and possible partial unfolding leading to reduced activity.[50] Conversely, a weakly adsorbed enzyme would be able to desorb from fabric surface to substrate solution showing higher activity similar to a free enzyme. Therefore, possible enzyme desorption from printed samples and resultant activity are assessed in next section.

Practical application scenarios of the enzyme printed fabrics would require them to retain activity during storage and usage under room atmospheric conditions. Therefore, activity of the printed samples were measured upon storing in similar conditions for a prolonged period of time (Figure 3b). Significant reduction of activity was observed over time and after three days from printing, retained about half of the activity. Nevertheless, the samples retained about 29–36% and 11–14% activity that of immediately after printing while stored for 15 and 30 days, respectively. Similar results of activity maintenance for days even after repeated use have been observed for printed lactate oxidase on polyvinyl chloride sheets.[45] Reason for activity reduction over time in room conditions might be related to structural vulnerability of enzymes due to dehydration.[55,56] Therefore, higher amount of humectant (for example, glycerol) should be used in ink formulation to retain activity while storing under such conditions;[36,37] alternatively, these need to be cold-stored upon printing. An elevated amount of glycerol can ensure better protein folding of lysozyme,[36] thus retaining better activity. In our previous work with lysozyme,[12] slightly better activity retention was shown at higher viscosity ranges (10–15 mPa s) and thus it might require further adjustment for activity optimization. Careful increment of viscosity can promote self-association among enzymes by replacing the surrounding water molecules and thus resulting in improved activity and printing performance.

2.5. Adsorption Stability

Enzymatic activity variation between the alkali, cutinase, and plasma-treated samples might have been influenced by their ink adsorption stability as discussed in previous sections. Therefore, it was essential to study the extent of possible lysozyme desorption from printed samples to the substrate solution. This would further provide indication about the prominent route of activity, that is, substrate diffusion or enzyme desorption and possible end use of such printed surfaces. Accordingly, printed fabrics were subjected to equal amount of buffer as substrate solution for same duration as of the activity assays. Amount of absorbed protein was then measured by electrokinetic or ζ-potential that determines electrostatic interactions between the fabric surface and immobilized proteins at various pH of streaming liquid.[57]

Following pretreatments, isoelectric point (IEP) of all samples was moved to lower pH values (Figure 2a), hence, indicating presence of hydroxyl, carboxyl, and carbonyl groups on their surface.[35] IEP was lowest for plasma treated fabrics possibly owing to higher amount of surface bound polar groups. After printing (Figure 2b), the samples became amphoteric in nature

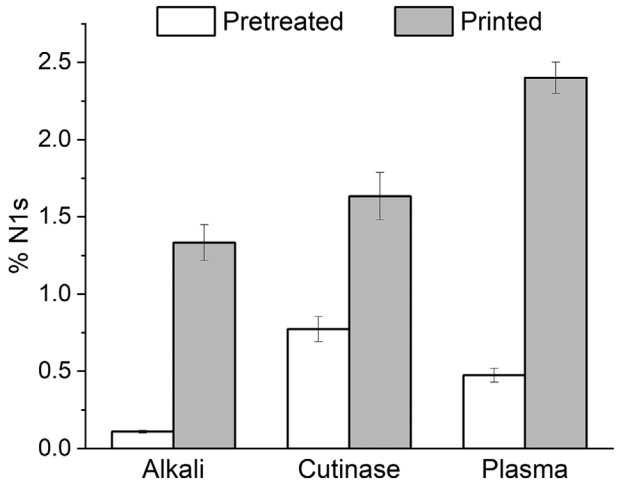

Figure 4. Nitrogen content found by XPS study of various pretreated PET fabrics (blank columns) and after inkjet printing of lysozyme on respective fabrics (grey columns).

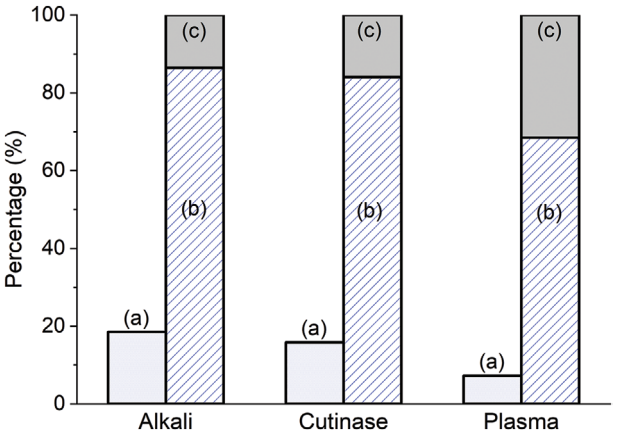

Figure 5. Protein number and lytic activity of lysozyme inkjet printed PET fabrics pretreated variously, after desorption in buffer solution; a) percentage of proteins found in desorbed buffer solution against total number of printed proteins, b) activity of enzymes found in desorbed buffer solution, and c) activity of enzymes, left on desorbed fabric; where, (b) and (c) are expressed as a percentage of respective sum.

due to increase of surface positive charges provided by amino acid groups of immobilized enzymes. Their IEP moved significantly toward that of lysozyme (pH 9–11) confirming well the presence of printed enzymes even under streaming conditions. All the printed samples showed similar ζ-potential values indicating almost equal amount of adsorbed enzyme. However, XPS results (**Figure 4**) indicated comparatively higher amount of enzymes were adsorbed on plasma treated fabrics. This difference might be due to the variation in measurement technique used by two instruments. XPS mostly works on outer surface layer (≈10 nm) of fabric in dry state and ζ-potential collects information from the whole fabric layer in wet-swollen state with higher accessibility. Considering the results from both analyses, it appeared that total amount of adsorbed enzyme was same for all samples, but for plasma treated fabrics, more toward outer surface of fiber. After desorption of the printed samples on buffer solution, IEP started to move significantly toward respective pretreated conditions, except plasma treated ones, indicating comparatively less protein desorption.

The findings of above ζ-potential studies was further validated by counting the number of proteins desorbed from the printed samples to buffer, along with measuring remaining enzymatic activity on buffer solution and on the desorbed out fabric surfaces as presented in **Figure 5**. Alkali (19%) and cutinase (16%) treated fabrics desorbed a significantly higher amount of printed proteins than plasma (7%) treated fabrics. Reason for such difference may be related to the protein adsorption mechanism of respective pretreated surfaces. Among two possible protein–surface interactions, that is, hydrophobic and electrostatic, lysozyme prefers the latter one with higher negative potential.[14] It has been explained with varying ionic nature of medium that system with electrostatic attraction between surface and lysozyme lowers desorption possibilities.[58] In case of alkali and cutinase treated samples with relatively lower hydrophilicity (Table 1), protein adsorption might have been partially governed by weaker hydrophobic interaction. This leads to higher activity in free solution mostly from desorbed enzymes (Figure 5), thus showing potential for controlled release related application. Conversely, surface characteristics of plasma modified samples (Figure 2a) provided better prospect of electrostatic interactions with possible ionic bond formation between the polar surface and lysozyme amino groups to result in stronger adsorption forces [29] that can be utilized for sensing applications. Additionally, it has been observed for higher protein concentrations, lysozyme may adsorb on a surface by forming multilayers [59] where the first layer was governed by electrostatic forces [50] and a second layer by weaker protein–protein interactions.[58] XPS results with higher N% on plasma-treated surface (Figure 4) indicate possible formation of such lysozyme multilayers. Thus, for these samples, only weakly bound enzyme molecules desorbed to buffer solution and resulted in comparatively lower activity (Figure 5).

Lysozyme desorbed on buffer solution majorly contributed (≈70–85%) to total found activity for all sample types (Figure 5). However, activity of the enzymes left on desorbed fabrics (after removing from buffer) were significantly higher on plasma-treated samples (32%) than on the alkali and cutinase treated ones (≈15%). As discussed earlier, this can be attributed to formation of lysozyme multilayers on plasma-treated surface and presence of a strongly adsorbed near-surface active layer even after desorption process. Activity of the buffer desorbed enzymes were about ten times lower than ink collected after jetting process. As discussed earlier, such high reduction of activity might have been caused by various immobilization phenomena subjected on the enzyme, for example, change of protein structure, shape and conformation, along with possible partial unfolding.[49–51] To recover the active state, enzymes would need to rearrange their structure upon desorption,[59] thus, activity of desorption-based system would be difficult to improve further. Conversely, less compromised activity can be expected from a surface retaining most enzymes even after desorption or washing process due to improved protein adsorption stability achieved through an appropriate physical and/or chemical surface modification. A balance between adsorption stability and activity between the three differently pretreated

fabrics can be further studied by improving hydrophilicity of the surfaces and introducing new compatible functional groups. These can be achieved probably by amplifying and altering the process parameters, for example,, higher chemical concentration and duration of alkali and cutinase treatments and using various gases (oxygen, nitrogen, and their mixtures) for plasma treatment process.

3. Conclusion

This study investigated several routes of PET fabric pretreatment to be inkjet printed with enzymes and thereby, retaining activity results with room for improvement. Pretreatment results showed that compared to alkaline and enzyme (cutinase)-based processes, air-atmospheric plasma treatment could improve PET wettability without much hampering of the other important physical properties. A successful ink formulation containing lysozyme was possible following previously developed optimization steps, and an increased range of linearity for enzyme concentration was observed upon printing. Proper immobilization of enzymes upon printing was confirmed with comparatively higher amount for plasma-treated fabric. Although, alkaline and cutinase-treated fabrics showed comparatively higher retained activity. Stability of printed enzymes to remain adsorbed on variously pretreated surfaces influenced the course of enzyme–substrate interaction and thereby, the resultant activity. This study demonstrates that along with considerations of physical and environmental impacts, various pretreatment approaches can be applied for inkjet printing of enzymes on synthetic fabrics depending on desired applications, for example, desorption after printing for controlled release, and retained adsorption for bio-sensing.

4. Experimental Section

Materials: The fabric used was a 100% PET in plain weave with weight and thickness of 150 GSM and 0.4–0.5 mm, respectively, thankfully provided by FOV Fabrics AB (Sweden). Lysozyme from chicken egg white (E.C. 3.2.1.17) and *Micrococcus lysodeikticus* cell (MLC) for enzyme activity assays were purchased from Alfa-Aesar (Germany) and Sigma–Aldrich (Germany), respectively. Industrial grade modified cutinase (EC 3.1.1.74) was kindly provided by Novozymes (product no. NS59038). Alcalase (EC 3.4.21.62) and calcium acetate were purchased from Merck KGaA. Bicinchoninic acid assay kit (BCA) was purchased from BioVision, Inc. (USA). All other chemicals were analytical grade and obtained from Sigma–Aldrich.

Pretreatment Process: Fabrics were pre-washed to remove any remaining dirt from production by using a non-ionic surfactant (1% w/w of Triton-X 100) for 30 min at 50 °C, followed by thorough rinsing with distilled water. Main pretreatment wash procedures were adopted from literature to ensure enhanced wettability with least effect on fabric weight and strength. Alkaline pretreatment was done by subjecting the fabrics in NaOH (1M) aqueous solution at 60 °C for 2 h under agitation, followed by extensive washing with distilled water and drying at room temperature.[17] Enzymatic pretreatment was achieved by washing the fabrics with 2% cutinase (on the weight of fabric) at 80 °C and pH 8.0 for 4 h,[60] followed by an after-wash procedure to remove remaining proteins from surface with alcalase (5 mg mL^{-1}) at 55 °C and pH 7.5 for 6 h.[61] Fabrics were then thoroughly rinsed with distilled water and dried at room temperature. Plasma treatment was performed on a machine called "Coating Star" manufactured by Ahlbrandt System (Germany). Atmospheric air was used as the gas and all the treatments were carried out at electrical power of 1 kW, frequency of 26 kHz, and inter-electrode distance of 1.5 mm.[23,24]

Fabric Characterization: Wettability of the fabrics were measured by using sessile drop method on an optical tensiometer (Attension Theta, Biolin Scientific, Sweden) with drop volume of 3 µL at room temperature. The water contact angle on three random position was measured immediately after landing of the drop on fabric surface. Wettability of the pretreated fabrics was improved by change of fiber surface morphology and introduction of hydrophilic functional group. The weight loss was measured as a percentage of $(W_1–W_2)/W_1$, where W_1 and W_2 denote the weight of samples before and after treatments at standard atmospheric condition. Tensile strength to rupture the fabrics was measured according to ISO 13 934/1 standard. Briefly, five dry strips from both warp and weft direction of each sample were subjected to a pretension of two Newton having a dimension of 20 cm × 5 cm on a semi-automatic electronic strength tester called Tensolab (Mesdan S.p.A). All samples were conditioned at standard atmosphere prior to testing.

Ink Formulation and Printing: An optimization of ink formulation was conducted following the same strategies suggested in our previous work [12] by varying amount of phosphate buffer, glycerol (as viscosity modifier and humectant), Triton X-100 (surfactant), and lysozyme. The optimized ink vehicle had a buffer, glycerol, and surfactant ratio of 70: 29.90: 0.10 (w/w) with pH adjusted to 7. Lysozyme (>23000 U/mg) was added to the ink vehicle to achieve various protein concentrations (see Result and Discussion section).

Printing was conducted with a high speed drop-on-demand piezoelectric industrial inkjet printhead (Konica Minolta, KM1024i) mounted on a custom-made printing setup (Urtidium B200, VdW-Consulting, Belgium). This printhead has a native resolution of 360 dpi with 1024 nozzles over a printing width of 72 mm. Inks were printed as solid rectangle on an area of 7 cm × 3 cm with the printhead set to frequency of 35 kHz and temperature of 25 °C. Printed fabrics were dried in room temperature for 1 h before proceeding for activity assays.

Enzyme Assay: Lytic activity of lysozyme was measured as a decrease in absorbance against substrate (MLC) solution at 450 nm for 4 min by a UV–vis spectrophotometer (Evolution 201, Thermo Scientific, USA). One active unit was defined as the amount of enzyme causing a decrease in absorbance of 0.001 per min. Activity units were calculated from the initial linear rate against a standard calibration curve covering a range of protein concentrations (see Result and Discussion section). Substrate solution of 0.01% (w/v) was prepared with 66 mM phosphate buffer adjusted to pH 6.5. Ink solution (0.10 mL) was added to substrate solution (2.5 mL) in a cuvette with 1 cm light path. Printed fabric were placed inside a modified cuvette system of same path length with equivalent amount of substrate solution. During activity measurement, cuvettes were equilibrated at 25 °C and kept under continuous magnetic stirring using a Peltier controller unit (Evolution, Thermo Scientific, USA).

Protein Quantification: Amount of proteins desorbed from printed fabrics after dipping in buffer solution for 4 min was counted by using BCA assay technique. A working solution was made by adding 50 parts of reagent A (sodium carbonate, sodium bicarbonate, bicinchoninic acid, and sodium tartrate in 0.1 M sodium hydroxide) and 1 part of reagent B (cupric sulfate). Desorbed buffer solution (0.1 mL) was added to the working solution (2.0 mL) and incubated at 37 °C for 30 min before cooling to room temperature. The concentration of proteins was measured by the corresponding absorbance at 562 nm against a constructed standard curve.

Scanning Electron Microscopy (SEM): SEM analysis was carried out using a FEI Quanta200 ESEM (Thermo Fisher Scientific) at low vacuum using water vapor as gaseous environment with an accelerating voltage of 15 kV.

X-ray Photoelectron Spectroscopy (XPS): XPS analysis (or, electron spectroscopy for chemical analysis) was performed on a PHI 5000 VersaProbe III instrument equipped with monochromated aluminium

source with photon energy of ≈1486.6 eV and beam size ≈800 µm in diameter. Electron neutraliser was used for charge compensation on insulated material. Survey scan was run in the range between 0 and 1330 eV with the pass energy 93.5 eV and energy step in the spectrum was 0.40 eV.

Atomic Force Microscopy (AFM): AFM was carried out by a Bruker Catalyst system on inverted Titanium-Silicon Eclipse Nikon microscope and Nikon DS-L3 camera. Analysis was performed in tapping mode at ambient air where a cantilever with a sharp tip was used to detect laser beam reflected to a photodetector and thereby, to generate a surface map of the specimen. All the samples were scanned at three different places to improve reproducibility. AFM images present height data on a scan size of 500 nm after first order flattening through NanoScope Analysis software (Bruker Corporation).

Zeta Potential: Zeta (ζ) potential and isoelectric point of the fabrics were measured using a SurPASS electrokinetic analyzer (Anton Paar, Austria). A pair of fabrics of same sample with an area of 10×20 mm^2 each was placed in the clamping cell to be separated by a spacer during formation of a streaming channel. A background electrolyte of 1 mM KCl solution was used and the pH was adjusted in the range of 3–10 with HCl (0.2 M) and NaOH (0.2 M). Measurement at a pH point took ≈15 s and different pieces of same sample were used at various pH to minimize the possibility of material leaching from fabric surface. Streaming current method and the Helmholtz–Smoluchowski equation were used to determine ζ-potential.[62]

Statistical Analysis: The OriginLab program was used for data and statistical analysis. All presented data points are the mean of at least three observations. The results mentioned as "significantly different" for $p < 0.05$, were obtained by the one-way analysis of variance and the Tukey test among two groups.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

biomaterials inkjet printing, lysozyme, polyethylene terephthalate (PET), pretreatment routes

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