Enzyme and Microbial Technology 46 (2010) 100–106

Contents lists available at ScienceDirect

Enzyme and Microbial Technology

journal homepage: www.elsevier.com/locate/emt

Plasma and electron-beam processes as pretreatments for enzymatic processes

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ARTICLE INFO

Article history:
Received 18 May 2009
Received in revised form 6 October 2009
Accepted 15 October 2009

Keywords:
Plasma
Electron-beam
Enzyme
Transglutaminase
Accessibility

ABSTRACT

Plasma generated by non-polymerizing gases (oxygen, air and nitrogen) and electron-beam irradiation under different atmospheres were studied as possible pretreatments to an enzymatic process with transglutaminase (TGase). The aim was to improve the accessibility of target groups of TGase present in wool fabrics to the enzyme, thanks to chemical etching or the removal of the epicuticle layer by physical etching, thus leading to increased formation of cross-links or incorporation of primary amine compounds.

For the plasma treatment, we found that oxygen promotes the cleavage of disulphide bonds by oxidation of sulphur species: a reduction of oxygen content in the gas plasma induced a reduction in oxidation yield, as shown by FTIR measurements. Conversely, nitrogen promoted a chemical etching reaction. The most significant effects were observed at high treatment power (400 W), where both cleavage of polymer chains and removal of the epicuticle layer were promoted.

Air plasma at high power was the most promising pretreatment to the enzymatic process. The modifications induced a good penetration of the enzyme into the fibre core and no significant changes in enzyme activity were observed in contact with the plasma-treated fabrics.

In contrast, by increasing the energy of the electrons in E-beam treatments no significant superficial modifications were observed. In fact, they promoted the cleavage of high-energy bond, such as S–S linkage, by enhancing depolymerization reaction.

1. Introduction

Low temperature glow discharge plasma is capable modifying the surface of polymer materials: the plasma species, generated by the electrical field, bombard and react on the surface of the substrate [1,2]. The bulk gas temperature in non-thermal plasma is relatively low (usually below 150 °C). As a result, the penetration of the activating species in plasma into polymeric materials is shallow and the interior of the material is only slightly affected [3]. Non-thermal plasma treatment can thus be used as a highly reactive tool for powerful but gentle modification of polymer materials (including thermally sensitive substrates), since it operates at low temperature and only changes the chemical and physical properties of the substrate surface [4]. Whilst the energy of the electrons in gas discharge plasmas is typically in the range of 1–10 electron volt (eV) [5], electron-beam (E-beam) accelerators generate electrons with a much higher energy, generally 300 keV to 12 MeV. These electrons may be used to modify polymer materials through direct electron-to-electron interactions. These interactions can create active species such as radicals, so there are different possible outcomes from the electron-beam irradiation of polymer materials, on the basis of the chosen operating conditions [5].

One of the most common textile applications of E-beam is the curing of fibre-reinforced composites [6,7]. Also, numerous papers have described the grafting of polymers, such as acrylates [8], onto fibres and fabrics.

Transglutaminases (EC2.3.1.13) are a large family of enzymes [9–11], which catalyse the posttranslational modification of proteins by forming covalent cross-links between glutamyl- and lysyl-residues (N^{ε}(γ-glutamyl)lysine isodipeptide), thus leading to increased protein stability and resistance to chemical and proteolytic degradation [12]. Transglutaminases (TGases) can also modify proteins by the covalent incorporation of compounds containing primary amines and if the polyamine is bi-functional, the cross-linking of proteins can also occur via N',N(γ-glutamyl) polyamine bridges [9,10].

In the textile industry, TGases have been shown to be capable to cross-link wool proteins. The additional isodipeptide bridges introduced between glutamine and lysine side residues resulted in increased tensile strength to wool yarns and fabrics (up to 15%), and such added cross-links also led to the remediation of damage caused by previous chemical processing (e.g. chlorine or

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doi:10.1016/j.enzmictec.2009.10.004

Table 1
Plasma treatment conditions.

Gas	Sample ID	Discharge power (W)	Treatment time (min)	$P_{degasing}$ (mbar)	P_{system} (mbar)
Oxygen (O_2) Nitrogen (N_2) Air (AIR)	WOEL_PLGAS_1	100	30	0.01	0.2
	WOEL_PLGAS_2	200	30	0.01	0.2
	WOEL_PLGAS_3	300	30	0.01	0.2
	WOEL_PLGAS_4	400	30	0.01	0.2

permonosulphuric acid) or by proteases, restoring fibre strength to the original values [13–15]. Wool garments treated with TGase were also found to have increased resistance to damage caused by domestic washing and wear and tear, and an increased longevity [14] due to increased cross-links, as well as reduced fabric felting propensity. The ability of TGases to graft active compounds or proteins onto wool fibres with a view to enhancing their functionality has also been demonstrated [16].

These studies indicated that the extent of enzymatic reaction and consequently of the effect on the properties of the textile material was highly dependent on the accessibility of the enzyme's target amino acids glutamine and lysine within the wool fibre proteins.

The authors wanted therefore to clarify whether fibre surface treatments such as plasma and E-beam could affect the effectiveness of a TGase treatment by improving the accessibility of target amino acids in wool to the enzyme or improving the penetration of the enzyme into the wool fibre cortex, thus accessing more sites for reaction.

Plasma treatments with different non-polymerizing gases (oxygen, air and nitrogen) and E-beam irradiation in air or nitrogen atmosphere were assessed as possible pretreatments to non-proteolytic enzymatic processes (such as TGase) to improve the accessibility of target groups in the wool proteins to the enzymes. Furthermore, possible synergistic effects achieved by applying the plasma/E-beam processes in series with the enzymatic treatment in terms of improved properties of the protein matrices were evaluated.

2. Experimental

2.1. Materials

2.1.1. Textile fabrics

The wool fabrics used were woven wool (98%) and elastane (2%) fabrics, with an area weight of 268 g/m^2, provided by Pecci (Italy) already industrially scoured.

2.1.2. Reagents

The microbial transglutaminase (mTGase) isolated from *Streptomyces mobaraense* was kindly supplied by Ajinomoto Co. Inc. (Tokyo, Japan) in a 1% (w/w) enzyme preparation containing 99% (w/w) of maltodextrins. The fluorescein isothiocyanate (FITC) was purchased from Invitrogen, UK. Except where otherwise stated, reagents were purchased from Sigma (Poole, UK).

2.2. Methods

2.2.1. Low temperature plasma (LTP) treatment

A radio-frequency capacitive system (developed by Next Technology Tecnotessile in collaboration with the University of Milan, Italy) operating at 13.56 kHz was used.

Three non-polymerizing gases were used: oxygen (O_2), nitrogen (N_2) and air (78% N_2; 21% O_2 mixture). Treatment conditions were as reported in Table 1.

2.2.2. Electron-beam irradiation (E-beam) process

A 300 keV electron-beam accelerator (Crosslinking, Sweden) was used in this work. The effects of varying the electron dosage were studied in two different reaction mediums (air and nitrogen). Treatment conditions were as reported in Table 2.

2.2.3. Spectroscopic techniques

Changes induced by LTP and E-beam processes on the chemical composition and superficial properties of the wool fibres were investigated by FTIR ATR spectrometry analysis (100 scans; 4 cm^{-1} resolution, Spectrum One PerkinElmer Apparatus) at the absorption frequencies reported in Table 3. The molar ratio of selected absorption bands [17] with respect to the Amide III stretching band were determined to investigate chemical changes induced by both irradiation processes in terms of (i) Bunte salt and cysteic acid formation which are dependent on the cleavage of disulphide linkages; (ii) cystine monoxide and cystine dioxide are indicated as intermediate of cystine oxidation reaction, and (iii) amino group generated by the cleavage of peptide linkage or by chemical etching of fabric surfaces.

Table 2
Electron-beam treatment conditions.

Gas	Sample ID	Dose (kGy)	Treatment time (s)
Air (AIR) Nitrogen (N_2)	WOEL_EB GAS_1	100	160
	WOEL_EB GAS_2	200	320
	WOEL_EB GAS_3	300	480
	WOEL_EB GAS_4	400	640

Changes to wool fibres were also studied through XPS analysis (PHI XPS Microprobe, Physical Electronic Inc., USA) to determine changes in the elemental composition of the fibres at surface level and whether changes in the ratios between carbon, nitrogen and oxygen were introduced by LTP and E-beam treatments.

2.2.4. Peroxide radicals concentration

The concentration of peroxide radicals after treatment was determined by DPPH (2,2-diphenyl-1-pycrylhydrazyl free radical) analysis as described elsewhere [18,19].

2.2.5. Fabric hydrophilicity

Hydrophilicity was determined using the TEFLON Test and the fabric wettability was determined by measuring the water imbibition time of 1 g of treated fabric in 1 l of water. The time required by the textile to absorb or soak water and go into the bottom of the cylinder is considered as the imbibition time.

2.2.6. Transglutaminase activity and accessibility of target residues within wool proteins

The influence of radicals generated during plasma and E-beam treatments on the activity of mTGase was determined using the Lorand assay [20] based on the TGase mediated incorporation of radiolabelled ^{14}C-putrescine. Fabric samples treated with plasma and E-beam in different atmospheres (in nitrogen, air and oxygen) were conditioned and sealed in an argon atmosphere to prevent radical quenching. As a control, a set of samples equally treated had all radicals formed by the treatments thermally quenched. Microbial TGase (100 μg/ml) was incubated with fabric samples at 37 °C in Tris–HCl buffer pH 7. Aliquots of the treatment liquor were extracted at 1 h incubation time and the mTGase activity was determined using a Packard Instruments Tri-Carb 300 Scintillation Analyser counter. Activity of any enzyme that could be absorbed onto the fabric was also determined. As a further control, the enzyme activity was measured after 1 h incubation in solution without any fabric sample and after incubation with an untreated fabric.

The amount of Gln residues in wool proteins that are available for reaction with mTGase was determined by measuring the amount of ^{14}C-putrescine incorporated by mTGase activity using a modification to the Lorand assay, in which the ^{14}C-putrescine was incorporated into wool fibres (2.5 mg). The fibres were incubated at 37 °C in 1 ml Tris–HCl buffer pH 7.0 containing 500 μg/ml mTGase and 50 μl of ^{14}C-putrescine stock solution. The radiolabelled wool protein was subsequently dissolved in 500 μl Soluene 350, and 200 μl of the solution were added to 2 ml of scintillation fluid, and counted using a scintillation counter as above.

Table 3
Characteristic IR absorbance frequencies.

Species	Structure	Wave number (cm^{-1})
NH bending	–N–H	1640
Cystine dioxide	–SO_2–S–	1127
Cystine monoxide	–SO–S–	1071
Cysteic acid	–SO_3^-	1040
Bunte salt	–S–SO_3^-	1020