6th Framework programme

Project no. **COOP-CT-2006-032877 (ENZUP)**

Project acronym: **ENZUP**

Project title: **ENZYMATIC UP-GRADING OF WOOL FIBRES**

Instrument: **Co-operative Research Project**

Thematic Priority: **In the "Horizontal Research Activities involving SMEs"**

PUBLISHABLE FINAL ACTIVITY REPORT

ENZYMATIC UP-GRADING OF WOOL FIBRES

Period covered: from **1 Oct. 2006 to 30 Sep. 2008** Date of preparation: **1 Dec. 2008**

Start date of project: **1 October 2006** Duration: **24 Months**

Project partner responsible author: **Dr Jinsong Shen**

Project partner organisation name: **De Montfort University**

Publishable executive summary

The market value of wool is limited by the fact that consumers place increasingly high demands on machine washability and soft handle. Felting shrinkage, which is a typical property of wool due to the configuration of the scales of the wool fibre, is a serious problem, especially during washing. Chlorination, followed by polymer deposition, is commonly used to modify the scales of wool fibres in order to confer shrink-resistance, but this involves major drawbacks with respect to contamination of the wastewater with adsorbable organic chlorides (AOX) and the environment.

Enzymatic treatment represents a very interesting alternative to chlorination. Recent studies suggest a reasonable possibility for improving wool shrink-resistance. There is, however, no commercial application so far, due to difficulty in controlling enzyme reactions on protein fibres. Proteolytic enzymes are known to penetrate and degrade the internal wool structure during processing, causing fibre damage. The ENZUP project targeted this problem. The proteases are being genetic engineered and modified to have large molecular sizes which will limit the enzyme penetration into the fibres. The degradation by proteases will only take place on the outer layers of wool fibres.

The project has developed chemically modified proteases with three different levels of molecular sizes. Production of the modified proteases has being optimised at the large scale in order to reduce the cost and also improve the efficiency of production. Fundamental studies have been undertaken to understand the effect of the enlarged molecular size of proteases on the treatment of wool and their surface modification. The knowledge and data for controlling enzymatic reactions on protein fibres has been obtained from the project to support the application of modified proteases in the bioprocesses including wool scouring and bio-finishing processes for wool fibres.

The technology for enlarging molecular size of proteases (subtilisin) has been developed further through genetic engineering approach in ENZUP project. The high molecular weight subtilisin is constructed based on the fusion of prosubtilisin E DNA sequence, from *Bacillus subtilis,* with a DNA sequence that codifies to an elastin-like polymer. Genetic modification of protease is now being optimised from lab scale to large scale production.

From the application of chemically and genetic modified proteases with high molecular weights on wool fibres, the results showed that these enzymes can be used for specific surface modification of protein materials including wool anti-felting treatment, reduction of pilling and low temperature dyeing without damaging wool fibres.

In addition, the chemical modification of proteases with Eudragit polymer can make the enzyme much stable and compatible with the most of detergent formulations. Therefore

the modification of proteases can lead to applications of proteases in washing detergent formulations for wool staining cleaning.

Further more, enzymatic treatment with modified proteases and subsequent enzymatic or polymer grafting techniques for functional finishing are being developed to provide either hydrophilic or hydrophobic property, antimicrobial, and better dyeability.

The ENZUP Project Consortium

The ENZUP project consortium comprises 12 partners representing academic and industrial organisations. The project is coordinated by Dr Jinsong Shen of Textile Engineering and Materials (TEAM) Research Group at De Montfort University in Leicester, UK.

- De Montfort University, Textile Engineering and Materials Research Group, The Gateway, Leicester, LE1 9BH, UK
  Contact: Dr. Jinsong (Jim) Shen, Coordinator
  Tel: +44-116-257550, Fax: +44-116-2577582, Email: jshen@dmu.ac.uk
  www.dmu.ac.uk/team

- Tints Enrich S. L., Carretera Molins De Rei 201, 08205 Sabadell , Spain
  Contact: Mr Sebastià Serra, Technical Director,
  Tel: +34-93-7104291, Fax: +34-93-7205316 Email: serrarof@tintsenrich.com
  www.tintsenrich.com

- Color-Center, S. S. A., Ptge. Marie Curie 3, 08223 Terrassa, Spain
  Contact: Mr Jaume Mir, Managing Director,
  Tel: +34-670 721174, Fax: +34-93-7863434, Email: jaume-mir@colorcenter.es
  www.colorcenter.es

- Lokateks, Skofja, D. O. O., Kidriceva 75, Skofja Loka, SI-4220, Slovenia
  Contact: Ms Tatjana Rihtaršič
  Tel : +386-4-5111164, Fax : +386-4-5111119, Email: tatjana.rihtarsic@lokateks.si
  www.lokateks.si

- Qualizyme Biotechnology, Leechgasse 55/8, 8010 Graz, Austria
  Contact: Dr Andreas Paar, Managing Director,
  Tel: +43-699-10362215, Fax +43-316-327194, Email: office@qulizyme.com
  www.qualizyme.com

- VOF Ovis Texla, Weverstraat 38, 1791 AE Den Burg-Texel, The Netherlands
  Contact: Mr Henk Broekman, Managing director,
  Tel: +31-653-239846, Fax: +31-222-310441, Email: texla@planet.nl
  www.texelana.nl

- James weaker VOF, Mercuriusplein1, 5971 LW, Grubbenvorst, The Netherlands
  Contact: Mr Chris M.H.G. Reutelingsperger, Managing director,
  Tel: +31-77-3278000, Fax: +31-77-3278005, Email: chris@james.nl
  www.james.nl

- Graz University of Technology, Dept. of Environmental Biotechnology, Petersgasse 12, A-8010 Graz, Austria
  Contact: Prof. Georg M. Guebitz
  Tel: + 43 316 873 8312 Fax: + 43 316 873 8815, E-mail: guebitz@tugraz.at
  www.tugraz.at

- University of Minho, Departamento de Engenharia Têxtil, 4800-058 Guimarães, Portugal
  Contact: Prof. Artur Cavaco-Paulo
  Tel. +351-253-510280, Fax. +351-253-510293, E-mail: artur@det.uminho.pt
  www.uminho.pt

- TNO Science and Industry, Innovative Materials Dept. De Rondom 1, 5612 AP Eindhoven, The Netherlands
  Contact: Dr. Herman Lenting
  Tel: + 31 40 2650384 E-mail: Herman.Lenting@tno.nl
  www.tno.nl

- Polytechnical University of Catalonia, Dept. d'Enginyeria Quimica, EUETIT, Colom, 1, 08222 Terrassa (Barcelona)Spain
  Contact: Dr. Tzanko Tzanov
  Tel. +34 93 739 87 61, Fax +34 93 739 8225, E-mail tzanko.tzanov@upc.edu
  www.upc.es

- University of Maribor, Institute of Engineering Materials and Design, Smetanova ulica 17, SI-2000 Maribor, Slovenia
  Contact: Dr Vanja Kokol,
  Tel: +386-2-2207896, Fax: +386-2-2207990, Email: Vanja.kokol@uni-mb.si
  www.uni-mb.si

Project website: http://www.dmu.ac.uk/faculties/art_and_design/research/team/enzup/index.jsp

Dissemination and use

There are 9 major knowledge generated from the ENZUP project as listed below. Two new technologies for chemical modification and genetic engineering of proteases enlarging their molecular sizes have been protected by two patents filed already by project partner UMinho.

	Exploitable Knowledge	Exploitable product or measure	Sector of application	Timetable for commercial use	Patent or other IPR protection	Owner & other Partner(s) involved
1	New, genetically engineered proteases	New enzymes	Enzyme industry	2008 -10	Patented	All industrial partners, UMinho, TUG
2	Bio-scouring processes of raw wool	Scouring auxiliaries, processes	Textile and wool industry	2008 -10	Secret know-how	All industrial partners, DMU
3	Low temperature dyeing for wool	Fabrics, garments, yarns and tops, processes	Textile and wool industry	2008 -10	Secret know-how	All industrial partners, UMB, TNO
4	Shrink-resist finishing	Machine washable wool	Textile and wool industry	2008 -10	Patent to be planned	All industrial partners, DMU, TNO
5	Detergent formulations based on modified or engineered proteases	Carpet cleaning agents	Textile and wool industry	2008 -10	Patent situation evaluated and decided not to file	All industrial partners, TNO
6	A range of new, modified proteases with different molecular sizes	New enzymes	Enzyme industry	2008 -09	Patented and Secret know-how	All industrial partners, DMU, UMinho, TUG
7	Production of new products of wool using bioprocess with proteases	Fibres, yarns and knitted fabrics	Textile industry	2009 -10	Secret know-how	All industrial partners,
8	A simultaneous bioprocess for wool using proteases and transglutaminase	Fabric and garments, processes	Textile and wool industry	2008 -09	Publishable	All industrial partners, UPC
9	Detergent formulations based on modified proteases	Fabric and garments, auxiliaries,	Textile and wool industry	2008 -10	Secret know-how	All industrial partners, TNO

Publishable results for using and dissemination the knowledge

(1) Genetic modification of subtilisin and their application

A new process for genetic modification of proteases was developed. The high molecular weight protease is constructed based on the fusion of prosubtilisin E DNA sequence, from *Bacillus subtilis,* with a DNA sequence that codifies to an elastin-like polymer. Engineered proteases show increased molecular weight which prevents their diffusion into protein materials. Thus, these enzymes can be used for specific surface modification of protein materials including antifelting treatment of wool without damaging wool fibres. In addition, these proteases will find application in detergents for wool based textiles where the enzyme degrades protein stains without damaging wool fibres. These results are of high interest for applications in textile industry.

Wool yarns were treated with both commercial and chimeric subtilisins. It was found that the commercial subtilisin is able to penetrate inside the wool cortex, resulting in damage of the fibre as expected due to its small size. But using the chimeric subtilisin, there was a significant reduction of felting, pilling and tensile strength loss of wool yarns since the hydrolysis was restricted to the cuticle layer of wool.

The results stated here are of great importance once it is reported for the first time the microbial production of a chimeric high molecular weight protease for wool surface hydrolysis. This novel process of enzymatic-controlled hydrolysis overcomes the unrestrained diffusion of enzymes and extended fibre damage which are the major obstacle on the use of enzymes for wool finishing applications.

(2) A simultaneous bioprocess for wool using proteases and transglutaminase

A bioprocess for machine washable wool, combining the advantages of both protease and transglutaminase in a simultaneous enzymatic treatment has been developed. This process reduced the felting tendency of woven wool fabrics by 9% at the expense of only 2% weight loss and tensile strength loss. In contrast to previously described protease-based processes for shrink resistant wool, the anti-felting properties achieved in the simultaneous enzymatic treatment produced insignificant fibre damage, confirmed also by scanning electron images of the fabrics.

(3) Strategies towards the Functionalization of proteases from *Bacillus subtilis* for Wool Finishing Applications

Subtilisin E is an alkaline serine protease secreted by the Gram positive bacterium *Bacillus subtilis* and widely used in industry as a biocatalyst for various processes. The most common

application of subtilisins is in laundry detergents. However, due to environmental concerns, the application of subtilisins to treat wool, is under study. There are some reports regarding the attempts to substitute the conventional chlorine treatment by an enzymatic process capable of providing the same characteristics to the fabric, like anti-shrinking and better uptake and fixation of the dyestuff. However, the degree of uncontrolled hydrolysis due to diffusion of the enzyme inside the wool fiber causes unacceptable losses of strength.

To overcome this fact, and taking advantage of the x-ray crystallographic structure, the subtilisin E has genetically been modified, increasing its molecular weight, to restrict the hydrolysis to the surface of the wool fibers. Therefore, three genetically modified enzymes with a molecular weight 2-fold to 4-fold higher than the native subtilisin E were produced and assessed for activity. The prokaryotic expression systems, pET25b (+), pET11b and pBAD C, were explored for the production of recombinant enzymes. The results demonstrated that regardless the expression system or strain used, chimeric subtilisins were not expressed with the correct folding. No active and soluble recombinant protein was recovered under the testing conditions. Despite this drawback, a novel approach was described to increase the molecular weight of subtilisin. The reported results are noteworthy and can indicate good guidelines for future work aiming at the solubilization of recombinant chimeric subtilisins.

(4) Large scale produced enzyme-modified wool fibres for machine-washable bed coverings

Because of its excellent characteristics, wool fibres are very much appreciated as basic material for garments, carpets and bed coverings. It is thought that application in medical environment will improve the experienced comfort level of patients creating thus an ideal recovery (well-being) environment. However, wool fibres have one major drawback which is its felting behaviour. For this reason, machine-based washing is impossible for products based on these fibres using normal washing programmes. This felting behaviour blockades the application of wool fibres in non-disposable products used in medical environments like hospitals and carehouses since machine-based cleaning is essential for hygienic reasons.

Wool fibres can be made machine-washable by applying the Hercosett process, which includes a chlorination step and the use of resin. This process is rather unfriendly for the environment and makes the fibres less flexible and therefore less comfortable in use. An alternative process is an enzyme-based one in which the outer surface of the wool scales is smoothened, especially at the edges. This process is making use of a modified proteolytic enzyme and has been verified on labscale.

(5) Modification of Esperase by covalent bonding to Eudragit polymers L 100 and S 100 for wool fibre surface treatment

The protease Esperase was modified by covalent bonding with two grades of a reversible soluble-insoluble co-polymer of methacrylic acid and methyl-methacrylate, namely Eudragit L 100 and Eudragit S 100. The optimum reaction conditions and washing protocol were investigated and it was found that Esperase modified with Eudragit L 100 showed greater activity than if modified with Eudragit S 100. This should be expected as there is a greater quantity of active sites, namely carboxyl groups, per mass of Eudragit L 100 in comparison with Eudragit S 100 to interact with the enzyme. Gel filtration confirmed that Eudragit L 100 covalently bonded to Esperase. Treatment of the modified Esperase on wool showed that the enzyme modified with Eudragit L 100 had greater activity towards the wool and appeared more effective in shrink resistant finishing.

Patents:

Portuguese patent nº 104124

PROCESSO DE TRATAMENTO DE FIBRAS DE ANIMAIS DE FORMA A AUMENTAR A RESISTÊNCIA DAS MESMAS AO ENCOLHIMENTO, Cavaco-Paulo A, Casal M, Araújo R, Silva C, Machado R.

Publications:

Journal Papers

Araújo R, Cavaco-Paulo A, Casal M (2008), Strategies towards the functionalization of Subtilisin E from *Bacillus subtilis* for wool finishing applications, *Eng. Life Sci.*, *8*(3), 238–249.

Araújo R, Casal M, Cavaco-Paulo A (2008), Application of enzymes for textile fibres processing (review), *Biocatalysis and Biotransformation*, 26(5): 332-349.

Araújo R, Machado R, Silva C, Cavaco-Paulo A, Casal M, A new recombination high molecular weight subtilisin for wool finishing application (to be submitted).

Gaffar Hossain Kh. M., Juan A R, Tzanov T (2008), Simultaneous protease and transglutaminase treatment for shrink resistance of wool, *Biocatal Biotransform*, 25 (5), 405-411.

Gaffar Hossain Kh M, González M D, Lozano G R and Tzanov T (2009), Multifunctional modification of wool using an enzymatic process in aqueous-organic media, *Journal of Biotechnology*, (accepted).

Gaffar Hossain Kh M, Daga J M, Riva A, Canal J M, Tzanov T (2009), Un proceso enzimático para lana resistente al encogimiento, *Quimica Textil,* 2008 (accepted).

Araújo R et al, Enzymatic Hydrolysis of Wool with a Genetically Modified Subtilisin E. (to be submitted).

Conference papers

Araújo R, Silva C, Machado R, Cabello C, Casal M, Cavaco-Paulo A, (2008), A new recombination high molecular weight subtilisin for wool finishing application, COST 868 Meeting in Varna, Bulgaria, September, 18-19, 2008.

Araújo R, Matamá T, Casal M and Cavaco-Paulo A (2007), Biomodification of textile fibres using enzymes, Micro-Biotec-2007 Congress Lisboa, Portugal , 2007.

Araújo R, Casal M and Cavaco-Paulo A. (2007), Functinalizing bacillus subtilis subtilisin E for wool finishing applications, COST Action 868 Biotechnical functionalization of renewable polymeric materials, First Annual Workshop, Graz, Austria, 2007.

Shen J (2008), Enzymatic shrink-resist process based on modified proteases for machine washable wool, *5th International Conference of Textile Research Division*, NRC, Cairo, Egypt. April 6-8, 2008.

Shen J (2007), Modification of proteases by covalent bonding to Eudragit polymers for wool fibre surface treatment, *5th International Conference of Textile Biotechnology*, Wuxi, China, October 24-27, 2007.

Lenting H (2007), The importancy of processing conditions for efficient use of enzyme technology in making machine washable wool fibres: where historical knowledge helps future, *5th International Conference of Textile Biotechnology*, Wuxi, China, October 24-27, 2007.

Shen J, Smith E, Farrand B, Zhang Q (2008), Development of functional surface coatings of wool fibre using sol gel or extracted protein resin, COST ACTION 868, Varna, Bulgaria, September 18-19, 2008.

Gaffar Hossain Kh M, Riva A, Canal J M, Tzanov T (2008), Enzymatic process for machine washable wool, *21st IFATCC Congress*, 6-9 May 2008, Barcelona, Spain.

Gaffar Hossain Kh. M, González M D, Lozano G R, and Tzanov T (2009), Enzymatic process for multifunctional protein fibres, *237th ACS National Meeting*, March 22-26, 2009. Salt Lake City, Utah, USA (Accepted).