Stato dell'arte dei trattamenti enzimatici su lana e poliestere

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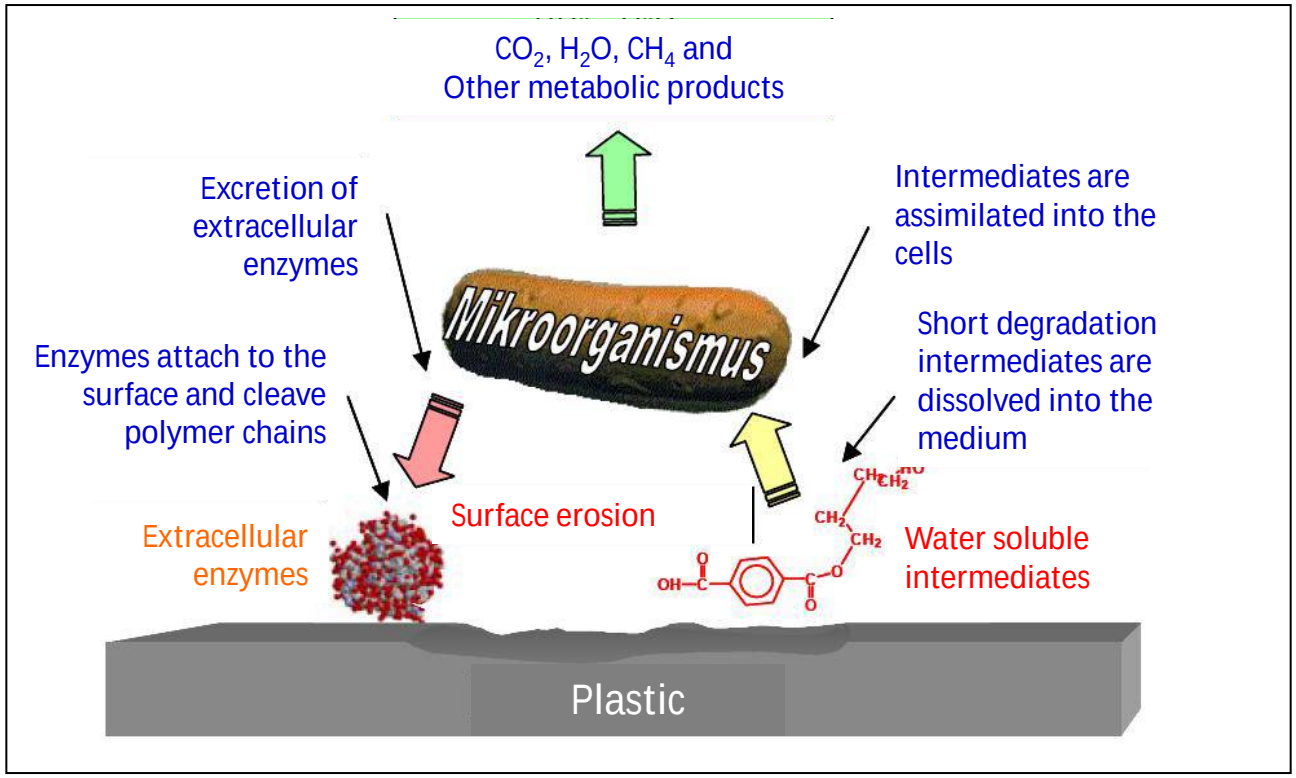
Busto Arsizio, 23 novembre 2012

BIODEGRADAZIONE DELLE PLASTICHE



Source: Mueller, Process Biochemistry, 41 (2006) 2125-2128

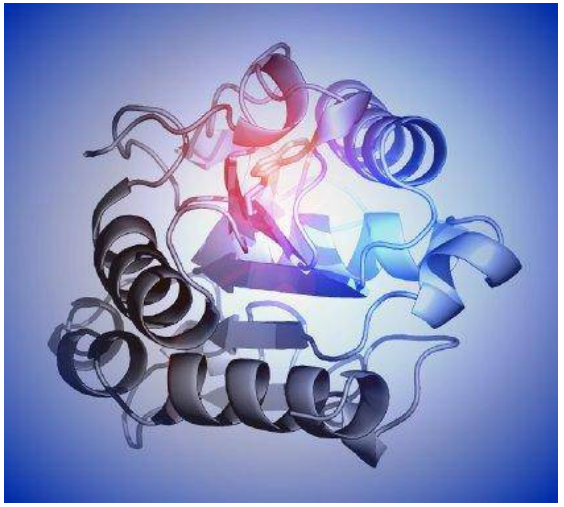
Depolimerizzazione del poliestere da parte di microorganismi in natura

POTENZIALITA' INDUSTRIALI DEGLI ENZIMI

- Gli enzimi non possono penetrare all'interno dei polimeri (o fibre) e la loro azione è limitata alla superficie del materiale

- Le proprietà superficiali del polimero possono cambiare mentre le proprietà di massa rimangono invariate (assenza di perdita di peso, mantenimento delle proprietà meccaniche, di mano, drappeggio, ecc.)

- L'idrolisi superficiale di legami estere del poliestere modifica le proprietà chimiche di superficie e porta alla formazione di nuovi gruppi funzionali disponibili per ulteriori reazioni

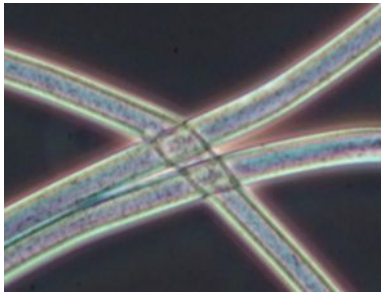


Cutinasi da *Fusarium solani pisi*

TRATTAMENTO ENZIMATICO DEL POLIESTERE

Il poliestere (polietilene tereftalato) è composto da:

- Glicole etilenico
- Acido tereftalico

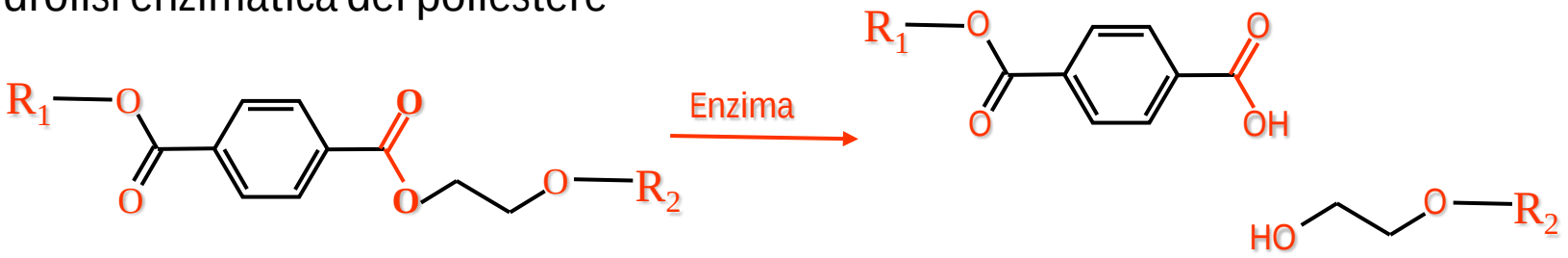


Fibre di poliestere

Enzimi attivi sul poliestere:

- Cutinasi (*Thermobifida fusca, Penicillium citrinum, Fusarium oxysporum, Fusarium solani pisi*)
- Altri (Lipasi, serina esterasi, nitrobenzil esterasi)

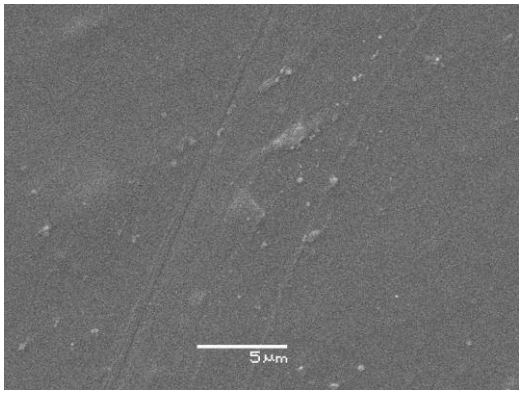
Idrolisi enzimatica del poliestere



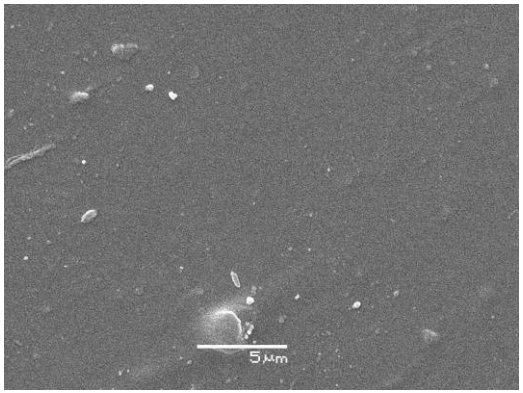
TECNOLOGIE ABILITANTI

Caratteristiche morfologiche

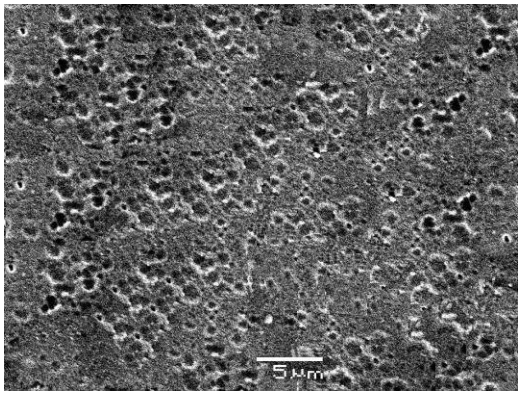
Non trattato



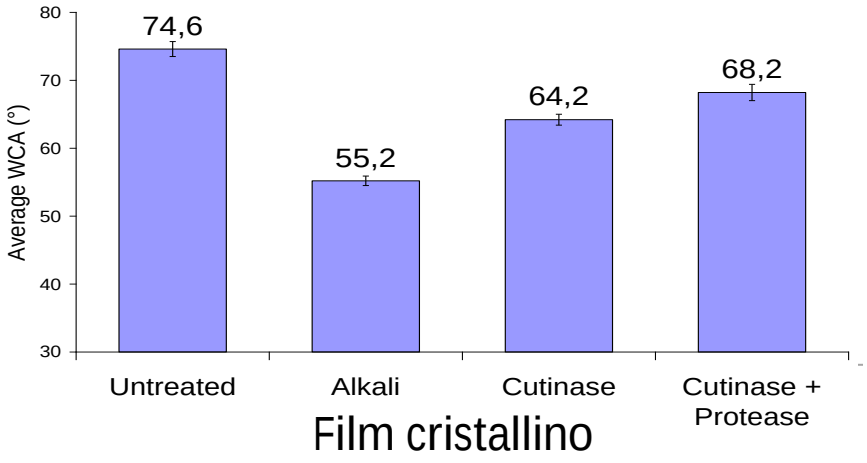
Trattato con Cutinasi



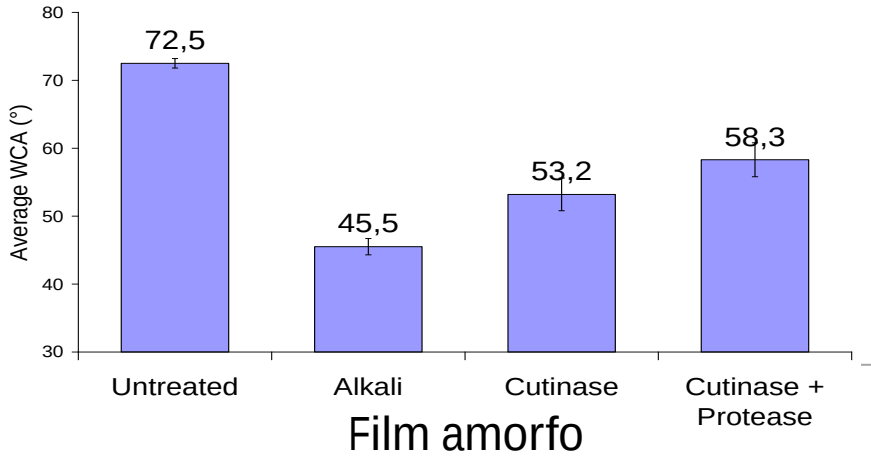
Trattato con NaOH



Proprietà di superficie (idrofilia)



Film cristallino



Film amorfo

LIMITAZIONI E POSSIBILI SVILUPPI FUTURI

La cutinasi ha le caratteristiche per diventare un enzima industriale per il trattamento delle fibre di poliestere:

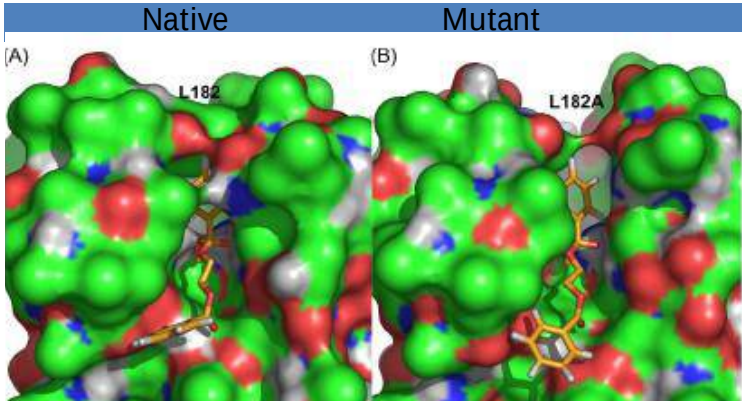
- Non richiede attivazione all'interfaccia col substrato
- È attiva sia su substrati solubili che allo stato solido

Limitazioni:

- Tempi di reazioni troppo lunghi (non compatibili con i processi tessili)
- Bassa efficienza catalitica
- Inerzia nei confronti dei materiali semi-cristallini (fibre)

Possibilità di sviluppo offerte dalle biotecnologie:

- Screening di microorgaismi e identificazione di nuovi enzimi
- Selezione di enzimi termostabili
- Sviluppo di nuovi enzimi con tecniche di ingegneria genetica



Source: Araujio et al., J. Biotechnol., 128 (2007) 849-857

LIMITAZIONI E POSSIBILI SVILUPPI FUTURI

Macromolecules

ARTICLE

pubs.acs.org/Macromolecules

Enzymatic Surface Hydrolysis of PET: Effect of Structural Diversity on Kinetic Properties of Cutinases from *Thermobifida*

Enrique Herrero Acero,[†] Doris Ribitsch,[†,*] Georg Steinkellner,[†] Karl Gruber,[†,‡] Katrin Greimel,[†] Inge Eiteljoerg,[†] Eva Trotscha,[†] Ren Wei,[§] Wolfgang Zimmermann,[§] Manfred Zinn,[⊥] Artur Cavaco-Paulo,[∥] Giuliano Freddi,[○] Helmut Schwab,[†,▽] and Georg Guebitz[†,#]

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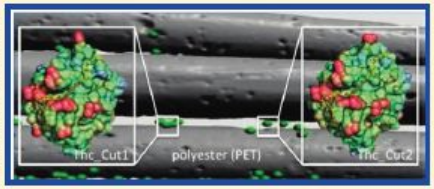
[‡]Institute of Molecular Biosciences, University of Graz, Graz, Austria
[§]Department of Microbiology and Bioprocess Technology, Institute of Biochemistry, University of Leipzig, Leipzig, Germany
[⊥]Laboratory for Biomaterials, Swiss Federal Laboratories for Materials Science and Technology (Empa), St. Gallen, Switzerland
[∥]Textile Engineering Department, University of Minho, Guimarães, Portugal
[○]Stazione Sperimentale per la Seta, Milano, Italy
[▽]Institute of Molecular Biotechnology, Graz University of Technology, Graz, Austria
[#]Institut for Environmental Biotechnology, Graz University of Technology, Graz, Austria

ABSTRACT: In this study cutinases from *Thermobifida cellulosilytica* DSM44535 (Thc_Cut1 and Thc_Cut2) and *Thermobifida fusca* DSM44342 (Thf42_Cut1) hydrolyzing poly(ethylene terephthalate) (PET) were successfully cloned and expressed in *E.coli* BL21-Gold(DE3). Their ability to hydrolyze PET was compared with other enzymes hydrolyzing natural polyesters, including the PHA depolymerase (ePhaZmd) from *Pseudomonas fluorescens* and two cutinases from *T. fusca* KW3. The three isolated *Thermobifida* cutinases are very similar (only a maximum of 18 amino acid differences) but yet had different kinetic parameters on soluble substrates. Their k_{cat} and K_m values on pNP−acetate were in the ranges 2.4−211.9 s^{-1} and 127−200 μM while on pNP−butyrate they showed k_{cat} and K_m values between 5.3 and 195.1 s^{-1} and between 1483 and 2133 μM. Thc_Cut1 released highest amounts of MHET and terephthalic acid from PET and bis(benzoyloxyethyl) terephthalate (3PET) with the highest concomitant increase in PET hydrophilicity as indicated by water contact angle (WCA) decreases. FTIR-ATR analysis revealed an increase in the crystallinity index A_{1340}/A_{1410} upon enzyme treatment and an increase of the amount of carboxylic and hydroxylic was measured using derivatization with 2-(bromomethyl)naphthalene. Modeling the covalently bound tetrahedral intermediate consisting of cutinase and 3PET indicated that the active site His-209 is in the proximity of the O of the substrate thus allowing hydrolysis. On the other hand, the models indicated that regions of Thc_Cut1 and Thc_Cut2 which differed in electrostatic and in hydrophobic surface properties were able to reach/interact with PET which may explain their different hydrolysis efficiencies.



Biocatalysis and Biotransformation, Month 2011; Early Online, 1–8

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healthcare

ORIGINAL ARTICLE

Characterization of a new cutinase from *Thermobifida alba* for PET-surface hydrolysis

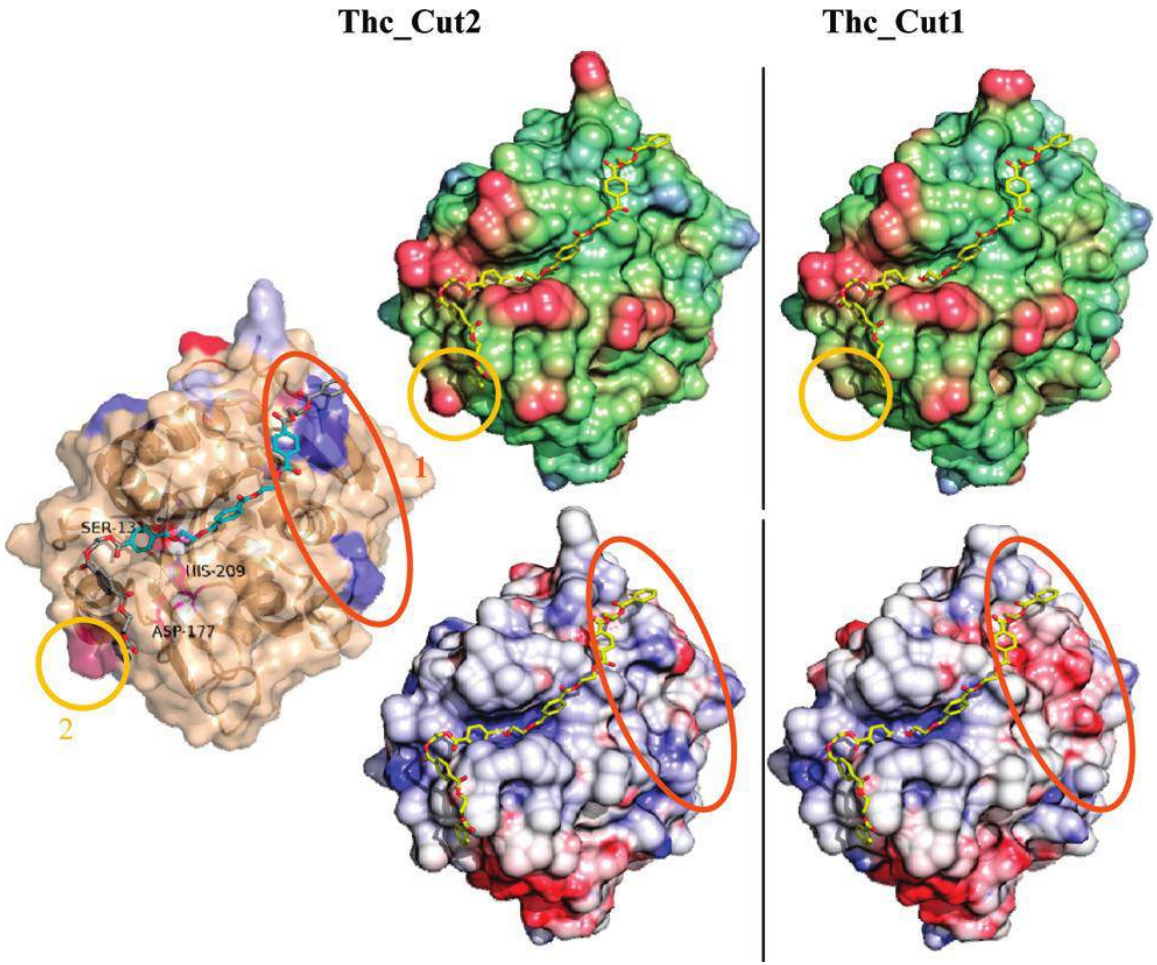
DORIS RIBITSCH[1], ENRIQUE HERRERO ACERO[1], KATRIN GREIMEL[1], INGE EITELJOERG[1], EVA TROTSCHA[1], GIULIANO FREDDI[2], HELMUT SCHWAB[1,3] & GEORG M. GUEBITZ[1,4]

[1]*ACIB, Petersgasse 14, Graz, Austria,* [2]*Stazione Sperimentale per la Seta, Milano, Italy,* [3]*Institute of Molecular Biotechnology, Graz University of Technology, Graz, Austria, and* [4]*Institute for Environmental Biotechnology, Graz University of Technology, Graz, Austria*

Abstract

A new cutinase from *Thermobifida alba* (Tha_Cut1) was cloned and characterized for polyethylene terephthalate (PET) hydrolysis. Tha_Cut1 showed a high degree of identity to a *T. cellulolysitica* cutinase with only four amino acid differences outside the active site area, according to modeling data. Yet, Tha_Cut1 was more active in terms of PET surface hydrolysis leading to considerable improvement in hydrophilicity quantified based on a decrease of the water contact angle from 87.7° to 45.0°. The introduction of new carboxyl groups was confirmed and measured after esterification with the fluorescent reagent alkyl bromide, 2-(bromomethyl) naphthalene (BrNP), resulting in a fluorescence emission intensity increase from 980 to 1420 a.u. On the soluble model substrates *p*-nitrophenyl acetate (PNPA) and *p*-nitrophenyl butyrate (PNPB), the cutinase showed *K*m values of 213 and 1933 µM and k_{cat} values of 2.72 and 6.03 s^{-1} respectively. The substrate specificity was investigated with bis(benzoyloxyethyl)terephthalate (3PET) and Tha_Cut1 was shown to release primarily 2-hydroxyethyl benzoate. This contrasts with the well-studied *Humicula insolens* cutinase which preferentially liberates terminal benzoic acid from 3PET.

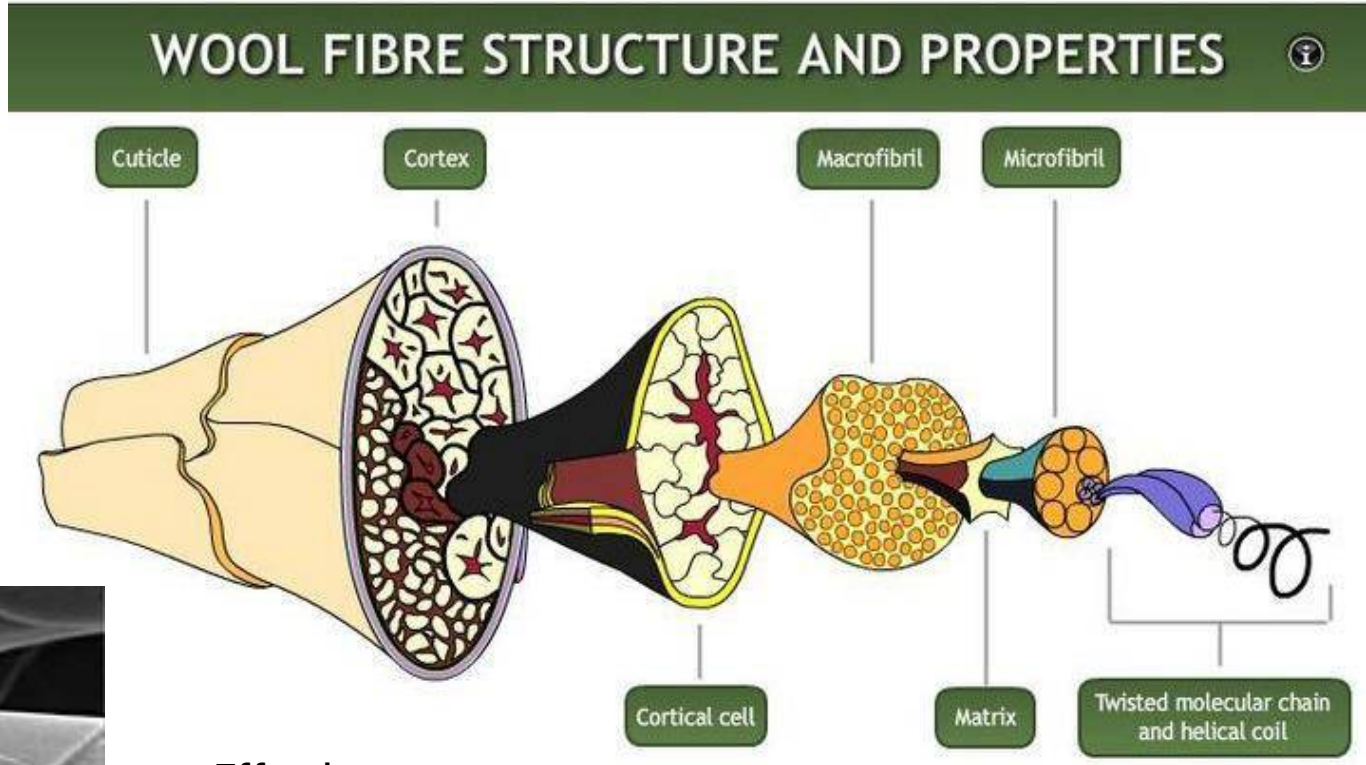
LIMITAZIONI E POSSIBILI SVILUPPI FUTURI

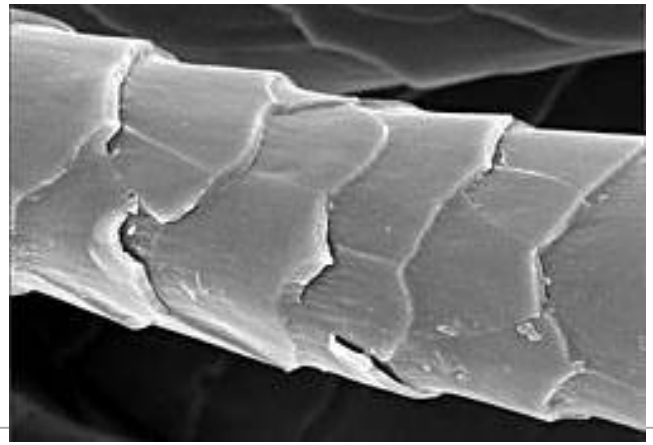


TRATTAMENTO ENZIMATICO DELLA LANA

Enzimi:

- Proteasi
- Cutinasi
- Transglutaminasi
- Tirosinasi
- Disulphide isomerasi

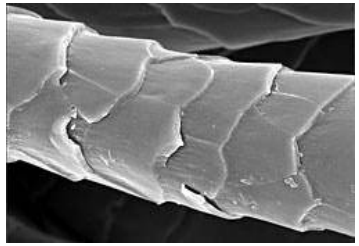


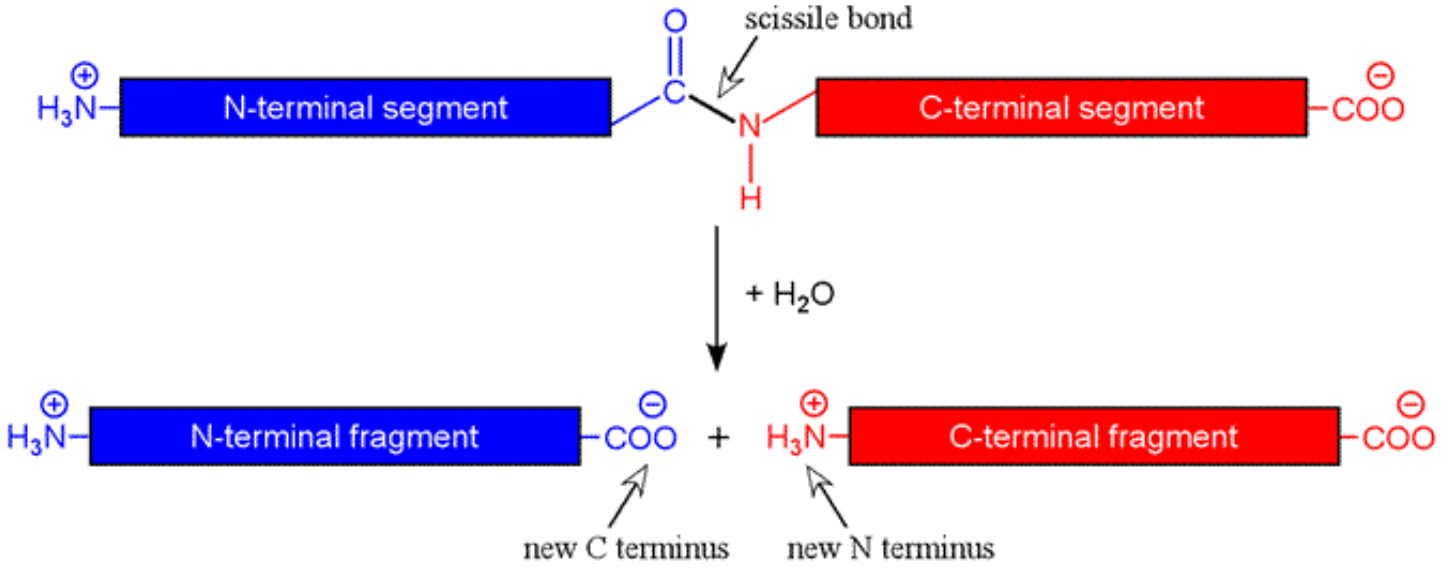


Effetti:

- Modificazione superficiale (antifeltrante)
- Tintura
- Finissaggio

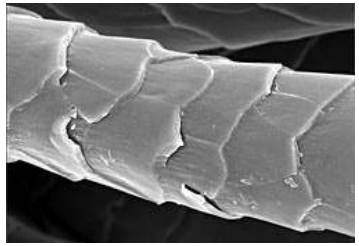
PROTEASI





Trattamento antifeltrante enzimatico: degradazione proteolitica

PROTEASI



Enzyme and Microbial Technology 47 (2010) 105–111

Contents lists available at ScienceDirect

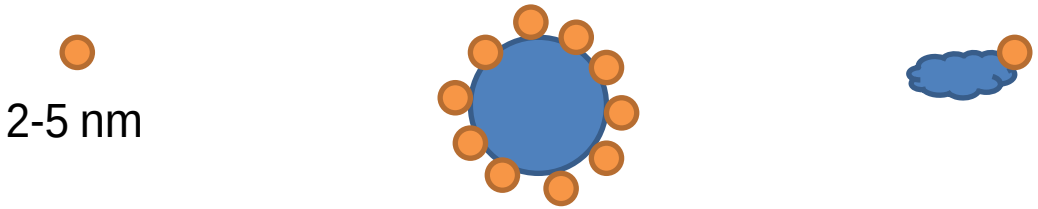
Enzyme and Microbial Technology

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

Covalent bonding of protease to different sized enteric polymers and their potential use in wool processing

Edward Smith [a], Marc Schroeder [b], Georg Guebitz [b], Jinsong Shen [a,*]

[a] Textile Engineering and Materials (TEAM) Research Group, De Montfort University, Leicester LE1 9BH, UK
[b] Department of Environmental Biotechnology, Graz University of Technology, Austria



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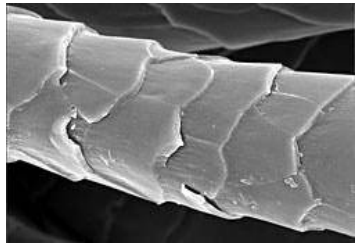
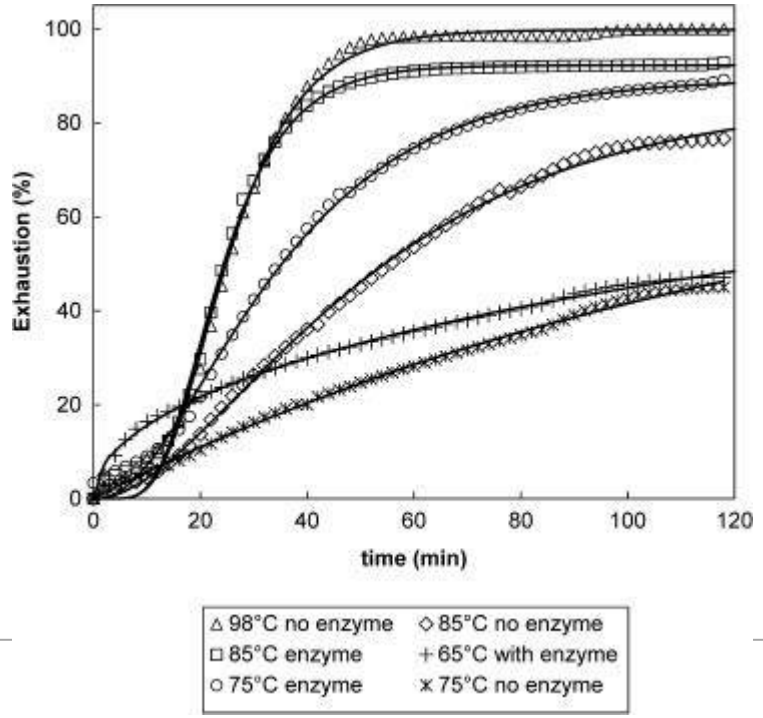


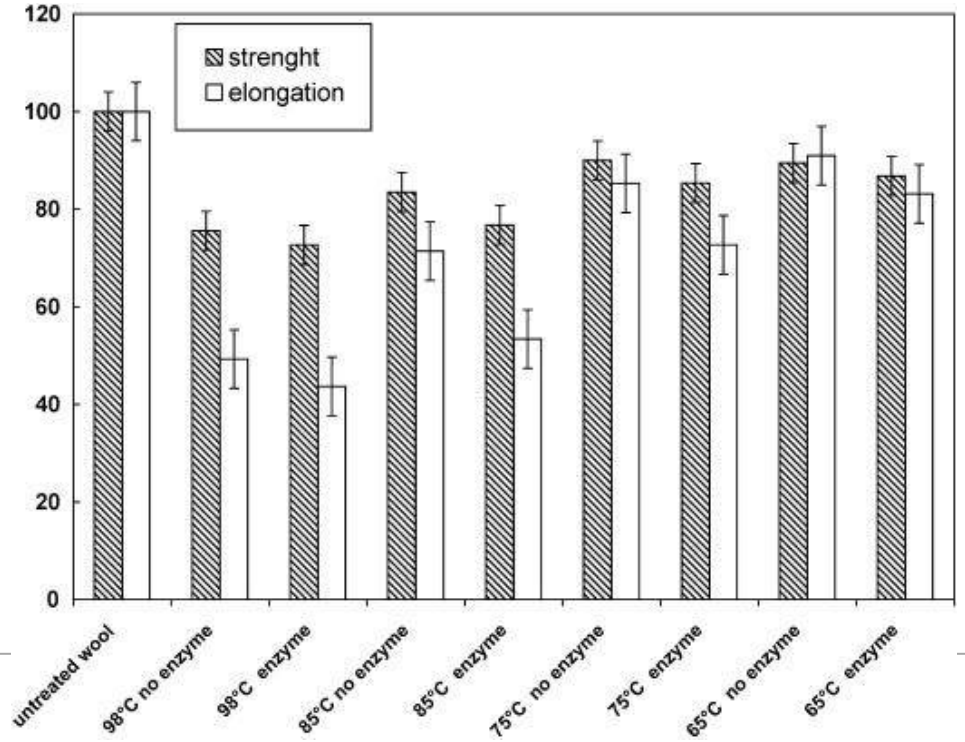
Eng. Life Sci. **2010**, *10*, No. 5, 474–479

Monica Periolatto[1]
Franco Ferrero[1]
Mirco Giansetti[1]
Raffaella Mossotti[2]
Riccardo Innocenti[2]

Research Article

Enzyme-aided wool dyeing with a neutral protease at reduced temperatures





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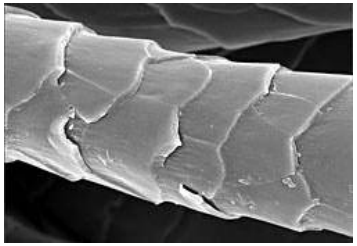

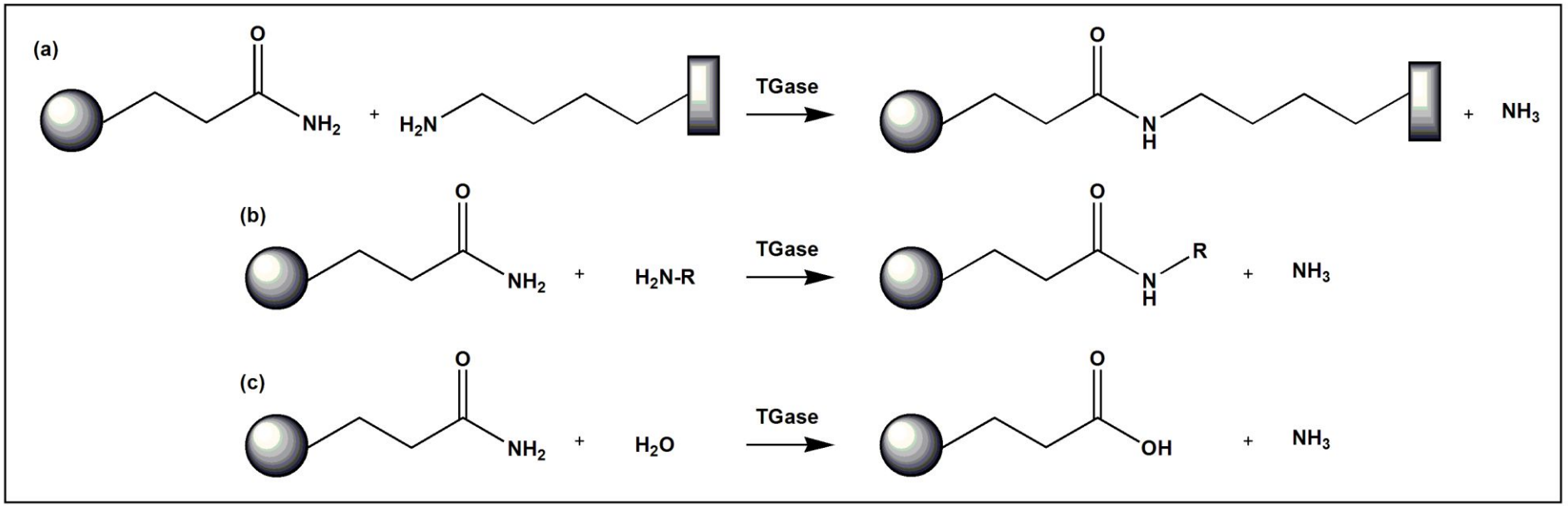

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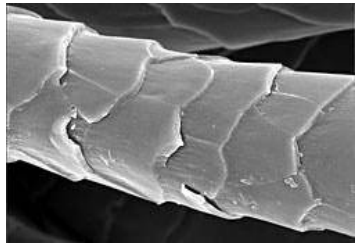

Biocatalysis and Biotransformation, September–October 2008; 26(5): 405–411

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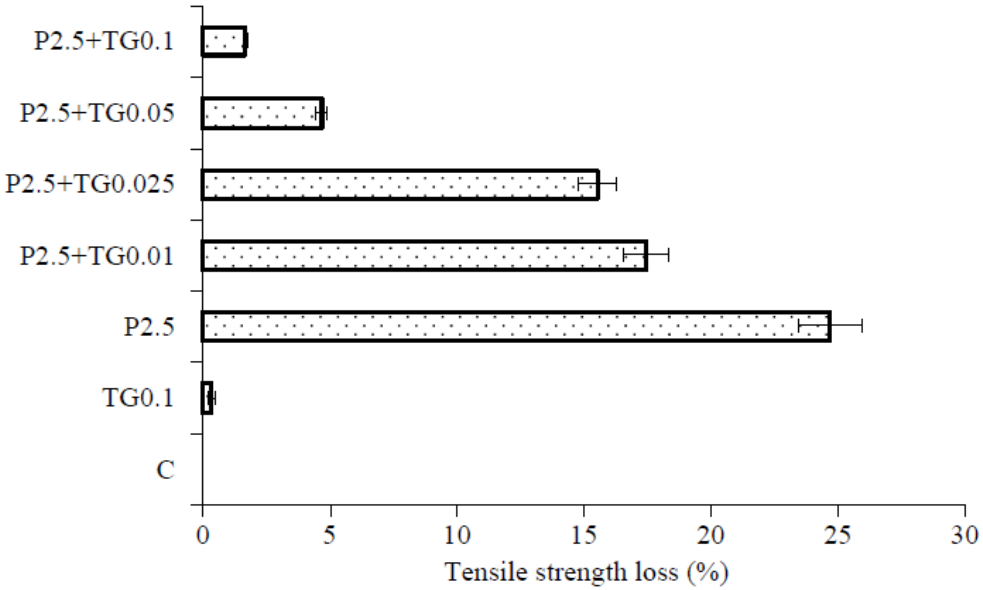
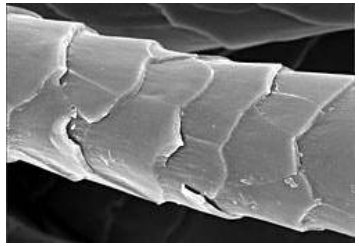
Simultaneous protease and transglutaminase treatment for shrink resistance of wool

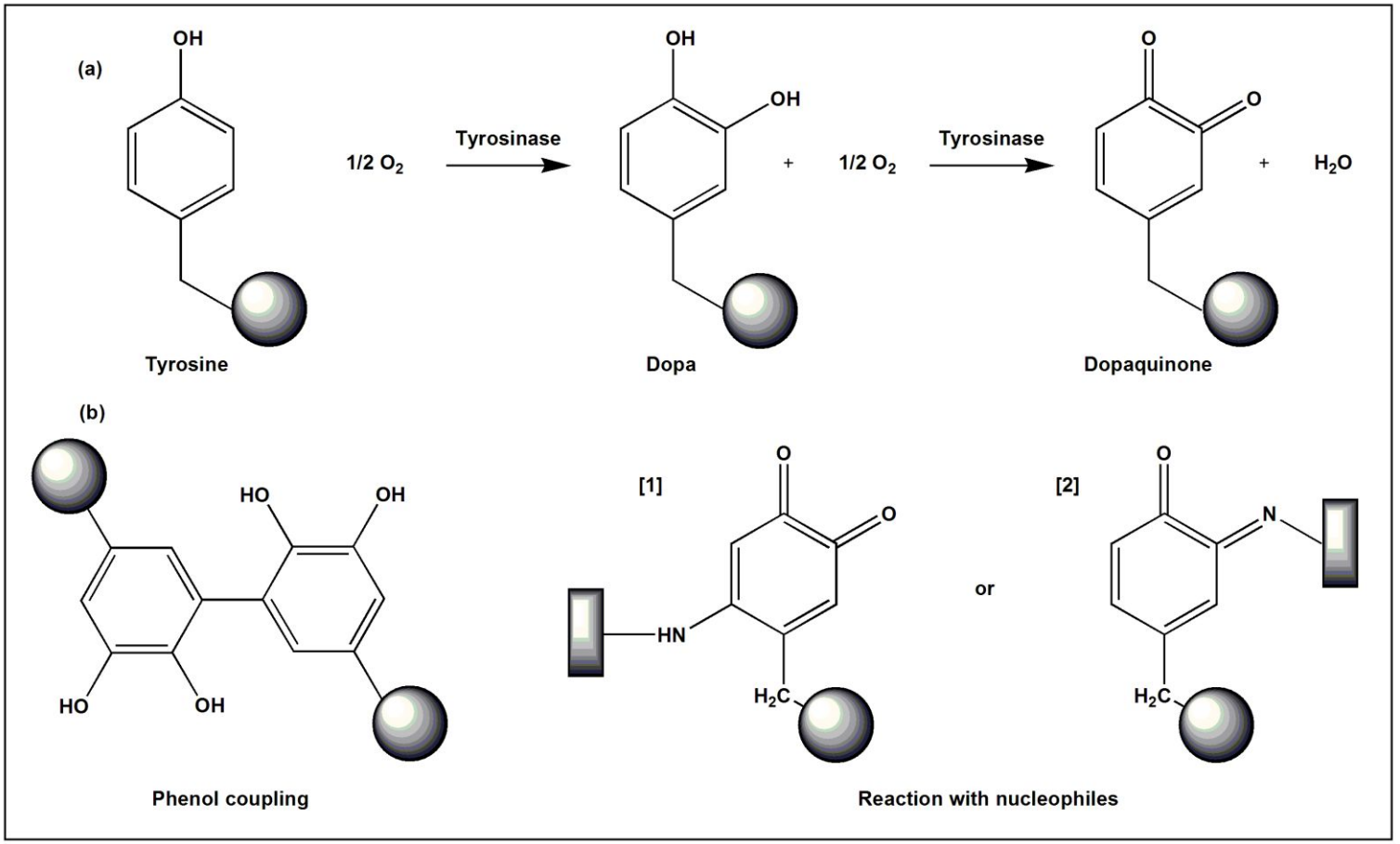
KH. M. GAFFAR HOSSAIN[1], ASCENSION RIVA JUAN[2], & TZANKO TZANOV[1]

[1]*Grup de Biotecnologia Molecular i Industrial and* [2]*Enginyeria Tèxtil i Paperera, Universitat Politècnica de Catalunya, Barcelona, Spain*

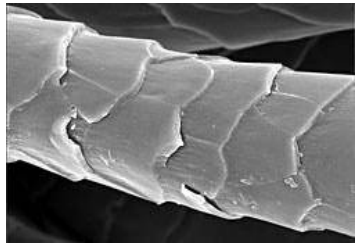


TIROSINASI




(a)
OH
1/2 O2
Tyrosinase
OH
OH
+
1/2 O2
Tyrosinase
O
O
+
H2O
Tyrosine
Dopa
Dopaquinone
(b)
HO
OH
HO
OH
Phenol coupling
[1]
O
O
HN
H2C
or
[2]
O
N
H2C
Reaction with nucleophiles

TIROSINASI



Tyrosinase catalysed coating of wool fibres with different protein-based bio-materials

SUZANA JUS[1, 2], VANJA KOKOL[2,*], GEORG M. GUEBITZ[1]

[1]*Technical University of Graz, Institute of Environmental Biotechnology, Petersgasse 12, A-8010 Graz, Austria*

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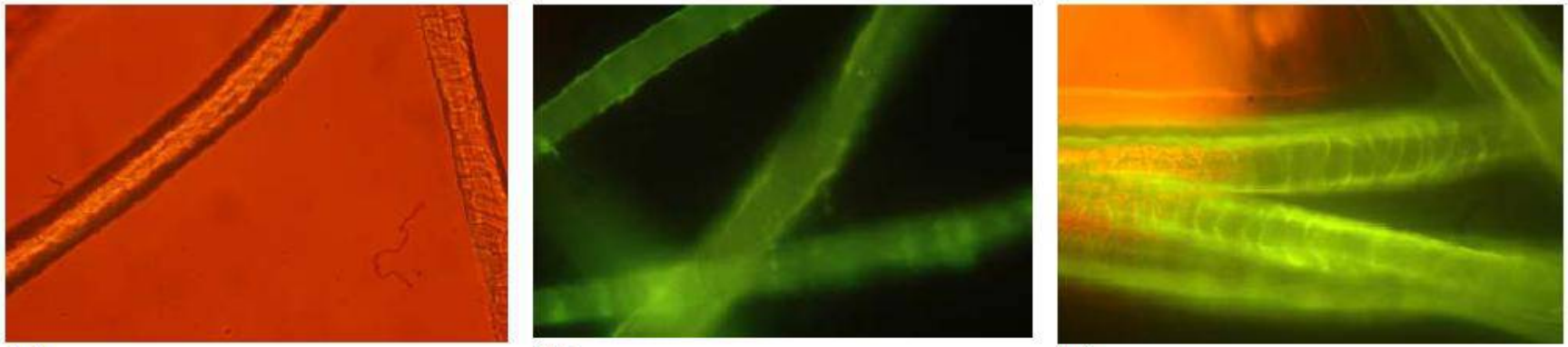
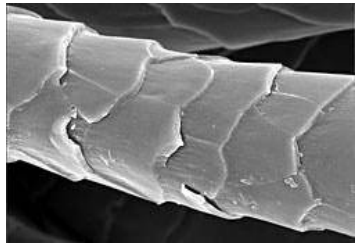


(a) (b) (c)

Fig. 8: Fluorescence micrographs of wool fibres treated with tyrosinase and FITC-labelled collagen (a) before treatment, (b) after enzyme treatment and (c) after washing

USO COMBINATO DI ENZIMI



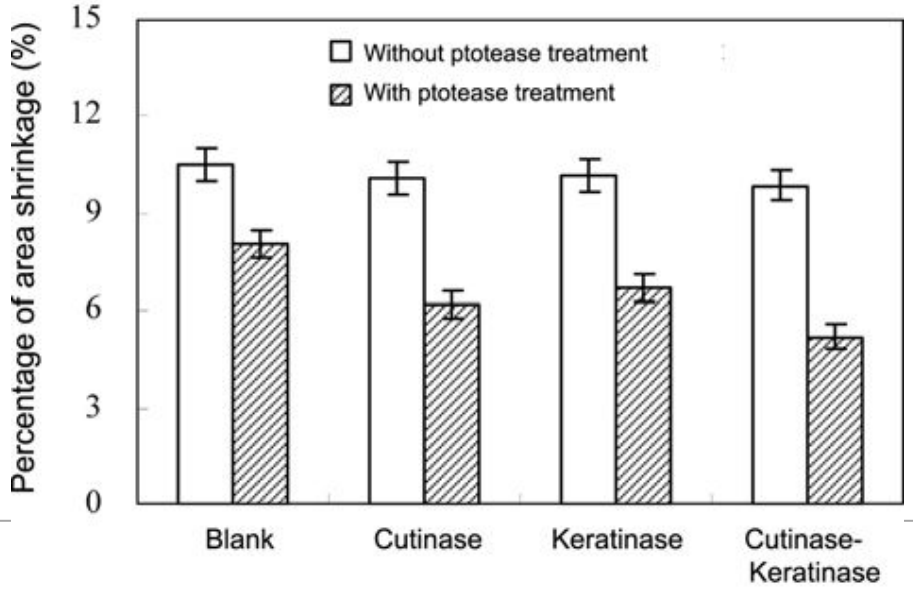
Fibers and Polymers 2011, Vol.12, No.6, 760-764 DOI 10.1007/s12221-011-0760-6

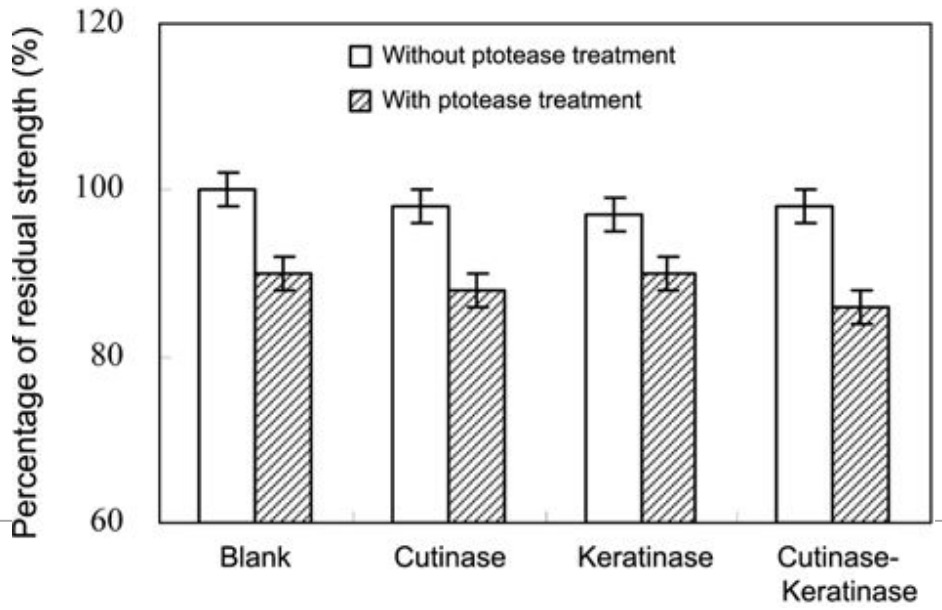
The Combined Use of Cutinase, Keratinase and Protease Treatments for Wool Bio-antifelting

Ping Wang, Qiang Wang, Li Cui, Murong Gao, and Xuerong Fan*

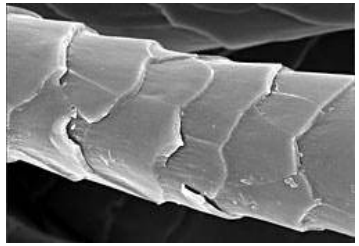
Key Laboratory of Science and Technology of Eco-Textile, Ministry of Education, Jiangnan University, Wuxi 214122, China

(Received September 11, 2010; Revised February 3, 2011; Accepted April 22, 2011)





PROTEIN DISULPHIDE ISOMERASE

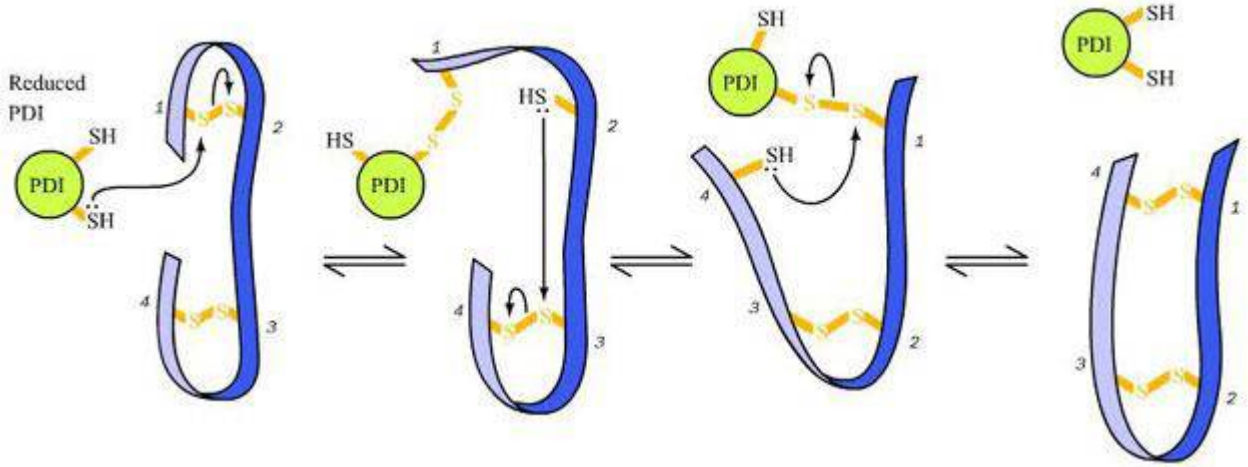


Appl Microbiol Biotechnol (2011) 90:1311–1321
DOI 10.1007/s00253-011-3194-6

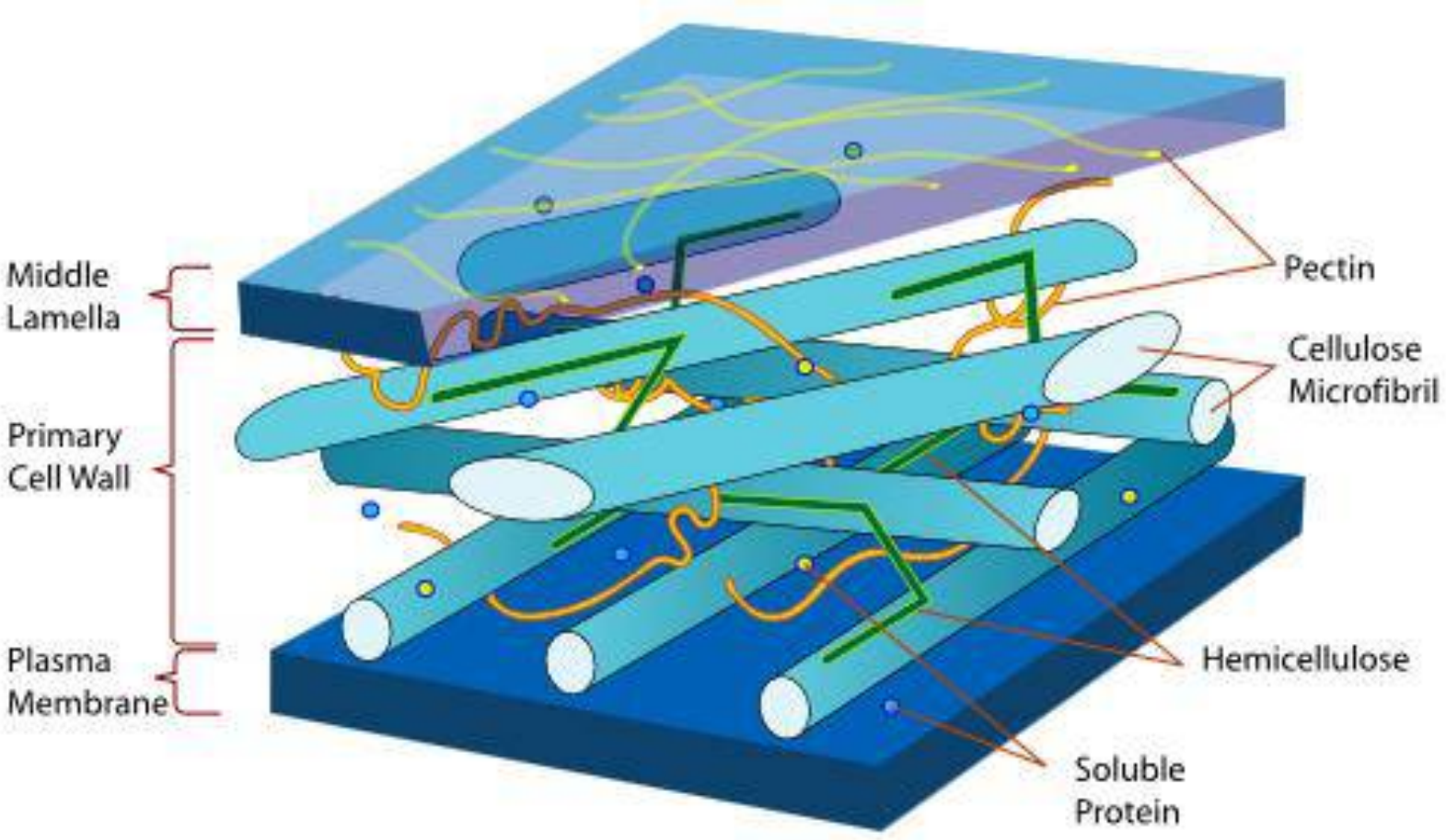
BIOTECHNOLOGICALLY RELEVANT ENZYMES AND PROTEINS

Protein disulphide isomerase-assisted functionalization of keratin-based matrices

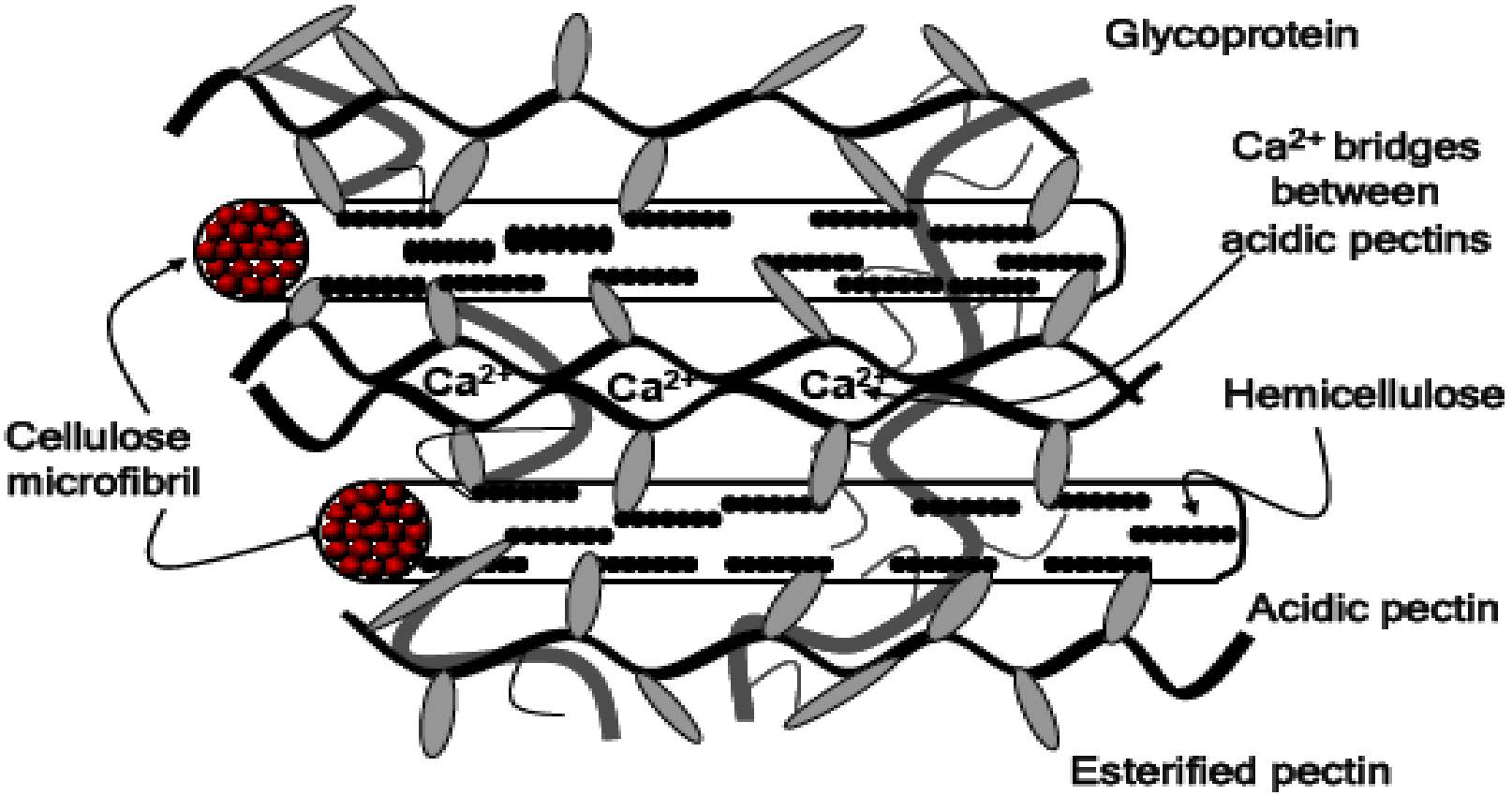
Margarida M. Fernandes · Andreia C. Gomes · Andreia Vasconcelos · Florentina-Daniela Munteanu · Tzanko Tzanov · Maria Sameiro T. Gonçalves · Nicole End · Kai-Uwe Schoening · Georg M. Guebitz · Artur Cavaco-Paulo



EMICELLULASI

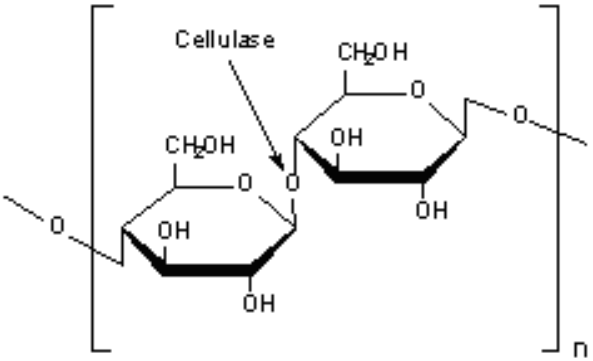


EMICELLULASI

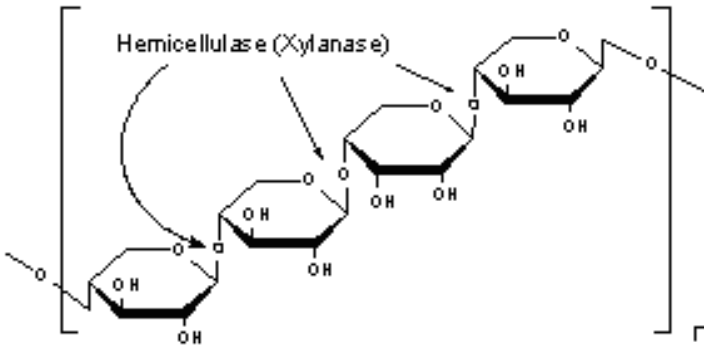


EMICELLULASI

Cellulose

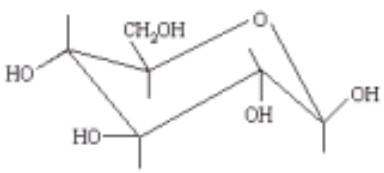


Hemicellulose

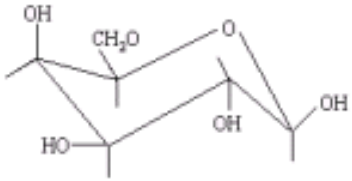


Xilanasi
Mannanasi
Arabinasi
Glicosidasi

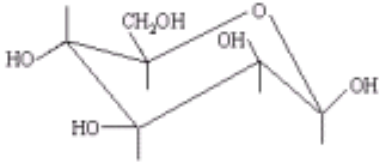
Cellulasi



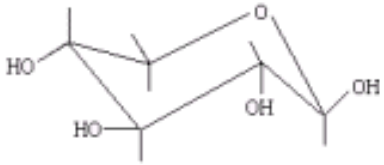
Glucose



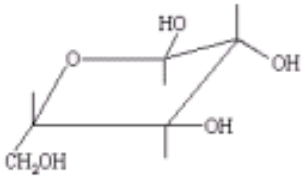
Galactose



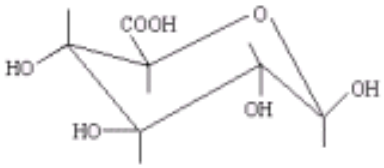
Manose



Xilose



Arabinose



Glicuronic Acid