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Pektinaze kot sredstvo za bioizkuhavanje

Pregledni znanstveni članek

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Izvleček

Encimi pektinaze v tekstilni industriji pridobivajo čedalje večji pomen. Uporablja se jih kot sredstvo za izkuhavanje bombaža, obdelava se imenuje bioizkuhavanje. Tako je že tudi na industrijski ravni razvitih več postopkov izkuhavanja, ki pa v praksi še niso povsem zaživeli. Obdelava poteka pri razmeroma nizki temperaturi v šibko kislem ali alkalnem mediju, kar je odvisno od vrste pektinaz. Pektinaze razcepijo pektin v vrhnji plasti bombažnih vlaken na manjše vodotopne molekule. Zaradi specifičnega delovanja pektinaz ne pride do poškodb celulozne verige. Z razgradnjo pektina se lažje odstranijo tudi ostale necelulozne snovi iz bombažnega vlakna. Vpojnost je zadostna za sledeče postopke obdelave. Odpadne vode niso obremenjene s škodljivimi kemikalijami.

Ključne besede: encimi, pektinaze, izkuhavanje, celulozna vlakna

Pectinases as agents for bioscouring

Review

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Abstract

The enzymes pectinases are gaining in importance in the textile industry. They are being used as an agent in scouring of cotton, such treatment of textile is called bioscouring. Several processes of scouring have been developed on industry level, however their use has not yet come to practical use. The treatment is carried out at considerably low temperatures in a weak acid or in alkaline medium, depending on the sort of pectinases. The pectinases decompose the pectin in the upper layer of cotton fibres to smaller water soluble molecules. Due to a specific activity of the pectinases, damages do not occur in the cellulose chains. Moreover, other non-cellulosic substances are easily removed from the cotton fibres by degradation of pectin. The absorption is sufficient for the following procedures of treatment. Waste waters are not charged with harmful chemicals.

Key words: enzymes, pectinases, scouring, cellulose fibres.

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1 Uvod

V zadnjih letih na številna industrijska področja prodira biotehnologija. Biotehnologija je aplikacija živih organizmov in njihovih komponent v industrijske postopke in proizvode. Leta 1981 je Evropska federacija za biotehnologijo definirala biotehnologijo kot združeno uporabo biokemije, mikrobiologije in kemičnega inženiringa z namenom izkoriščanja biokultur [1]. Biotehnologija ima korenine že daleč v zgodovini. Že v prvi polovici 19. stoletja so encime uporabljali pri pripravi pijač. Neizmeren napredek industrijske biotehnologije v zadnjih dvajsetih letih, predvsem v molekularni biologiji, proteinskem inženiringu in fermentacijski tehno-

1 Introduction

In the last few years biotechnology has been making its way into many areas of industry. Biotechnology is the application of life organisms and their components into industrial processes and products. In 1981 the European Federation of Biotechnology defined biotechnology as an applied use of biochemistry, microbiology and chemical engineering with the purpose of exploiting biocultures [1]. Biotechnology has its roots further back in history. In the first half of the 19th century enzymes were used in preparing drinks. The immense progress of industrial biotechnology in the last twenty years, especially in molecular biology, protein engineering and fermentation technology, enhanced the development of new uses of enzymes in the food industry, the use spread into the areas of detergents, paper and leather industry, natural polymer modification, organic chemical synthesis, diagnostics … In the last few years the use of enzymes experienced an increase in the textile industry as well. Today it offers a wide variety of alternative, environment and fibre friendly procedures which are replacing or improving the existing classical technological procedures. Different researches in the textile biotechnology have proven that enzymes can be efficient in treating natural cellulose fibres. Cellulases, proteases, amylases, catalases, pectinases, peroxidases and lactases are the enzymes that can replace aggressive chemicals [2].

2 Cotton fibre

A mature cotton fibre is composed of several concentric layers and a central area called lumen. A cuticle, a primary cell wall, intermediary wall as well as secondary cell wall follow each other from the outer to the inner part of the fibre. The whole cotton fibre contains 88 to 96.5 % of cellulose, the rest are noncellulosic substances, called incruste [3, 4]. Pectins, waxes, proteins, minerals and other organic substances are classified as noncellulosic substances. The larger part of these substances is found in the cuticle and the primary cell wall. Their quantity decreases as we proceed towards the secondary cell. During the growth of the fi-

logiji, je pospešil razvoj novih uporab encimov v živilski industriji, uporaba pa se je razširila tudi na področje detergentov, papirne in usnjarske industrije, naravne polimerne modifikacije, organske kemične sinteze, diagnosticiranja … V zadnjih letih je uporaba encimov doživela velik vzpon tudi v tekstilni industriji. Danes nudi široko področje alternativnih, okolju in vlaknom prijaznih postopkov, ki zamenjujejo ali izboljšujejo klasične tehnološke postopke. Različne raziskave v tekstilni biotehnologiji so dokazale, da so encimi lahko učinkoviti pri obdelavi naravnih celuloznih vlaken. Celulaze, proteaze, amilaze, katalaze, pektinaze, peroksidaze in lakaze so encimi, ki lahko nadomestijo agresivne kemikalije [2].

2 Bombažno vlakno

Zrelo bombažno vlakno sestavlja več koncentričnih plasti in sredinski prostor, imenovan lumen. Od zunaj navznoter si tako sledijo kutikula, primarna celična stena, vmesna stena in sekundarna celična stena. Celotno bombažno vlakno vsebuje od 88 do 96,5 % celuloze, ostalo so različne necelulozne snovi, ki se s skupnim imenom imenujejo inkrusti [3, 4]. Med necelulozne snovi se uvrščajo pektini, voski, proteini in minerali ter ostale organske snovi. Večji del teh snovi se nahaja v kutikuli in primarni celični steni. Proti sekundarni celični steni se njihova količina manjša. Necelulozne snovi, predvsem voski, ščitijo vlakna med rastjo pred izgubo vode, insekti in drugimi zunanjimi vplivi, ki bi vlakna lahko poškodovali. Ščitijo pa jih tudi pred mehanskimi poškodbami med predelavo. Pektin, ki ga je v bombažnih vlaknih od 0,4 do 1,2 %, deluje kot vezivo – lepilo med celuloznimi in necelulozni snovmi. Z njegovo odstranitvijo se lažje odstranijo tudi vse ostale necelulozne snovi. Za dobro učinkovitost beljenja, barvanja in drugih obdelav je namreč potrebno necelulozne snovi odstraniti.

Danes uveljavljeni postopki bioizkuhavanja temeljijo na razgradnji pektina z encimi pektinazami. Zato bomo v članku podrobno opisali zgradbo pektina in delovanje pektinaz.

2.1 Pektinske substance

Pektinske substance so generično poimenovane kompleksne polisaharidne makromolekule [5] z veliko in zelo različno molekulsko maso, negativnim nabojem ter kislim značajem. Primarno verigo sestavljajo z α-(1,4) vezjo povezane molekule α-D-galakturonske kisline. Stranske verige vsebujejo tudi molekule L-ramnoze, arabinoze, galaktoze in ksiloze, ki so vezane z glavno verigo preko prvega in drugega ogljikovega atoma. Strukturno formulo primarne verige pektina – poligalakturonske kisline prikazuje slika 1.

Homogalakturonski del polimera se imenuje »linearno« področje, področje bogato s stranskimi verigami pa »razvejano« področje [6]. Shematska predstavitev gladkih in razvejanih področij je prikazana na sliki 2.

Karboksilne skupine galakturonskih kislin so delno zaestrene z

bres noncellulosic substances, especially waxes, protect them against the loss of water, insects and other outside influences that might damage the fibres. Furthermore, they also protect them against mechanical damage that can occur as a result of processing. Pectin, there is 0.4 to 1.2 % of pectin in cotton fibres, acts as an adhesive, a glue between the cellulose and noncellulosic substances. By removing pectin, it is easier to remove all other noncellulosic substances. For bleaching, dyeing and other processing to be as effective as possible, noncellulosic substances need to be removed.

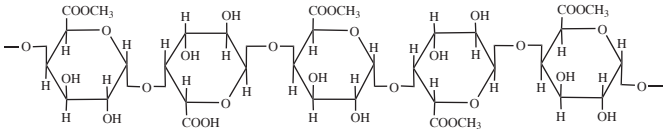
The processes of bioscouring that are in use today are based on the decomposition of pectin by the enzymes called pectinases. Therefore, the structure of pectin as well as the activity of pectinases will be closely examined in the article.

2.1 Pectin substances

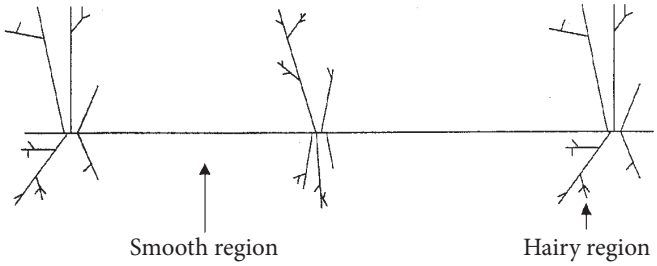
Pectin substances are generically called the complex polysaccharide macromolecules [5] with high and varying molecular mass. They are negatively charged and acidic. The primary chain is composed with α-(1,4) linked molecules of α-D-galacturonic acid. The side chains also contain molecules of L-rhamnose, arabinose, galactose and xylose that are connected to the main chain through their first and the second carbon atom. The structural formula of the primary chain of pectin – the polygalacturonic acid is shown in picture 1.

The homogalacturonan parts of the polymer are referred as »smooth« regions while the rhamnose-rich zones are called »hairy« regions [6]. The schematic representation of the »smooth« and the »hairy« regions is shown in picture 2.

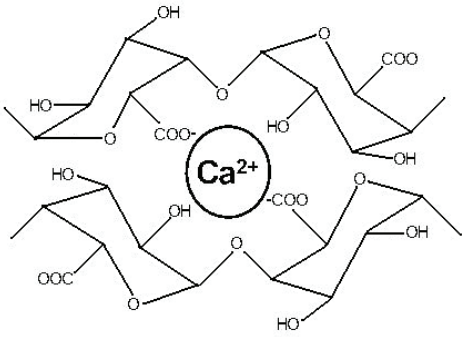
The carboxyl groups of galacturonic acid are partially esterified by methyl groups and partially or completely neutralized by calcium, potassium, magnesium, iron, ammonium or other ions. Some of the hydroxyl groups on the second and the third carbon atom can be acetylated [7–9]. Molecules with an increased number of negatively charged groups are straighter than esterified ones and therefore more likely to form a Ca^{2+} bridge. The phenomenon of the Ca^{2+} bridge, shown in picture 3, is demonstrated as the egg box model as proposed by Grant et al [10]. With the help of electrostatic interactions



Picture 1: Structural formula of the polygalacturonic acid.



Picture 2: Schematic structure of »smooth« and »hairy« regions of pectin [6].



Picture 3: Egg box model showing bondining of pectins with Ca^{2+} ions [12].

metilnimi skupinami in delno ali v celoti nevtralizirane s kalcijevimi, kalijevimi, magnezijevimi, železovimi, amonijevimi ali drugimi ioni. Nekatere hidroksilne skupine na drugem in tretjem ogljikovem atomu pa so lahko acetilirane [7–9]. Molekule z večjim številom negativno nabitih skupin so bolj ravne kot molekule z zaestrenimi skupinami in se v večji meri povežejo z ioni Ca^{2+}. Pojav povezovanja z ioni Ca^{2+}, ki je prikazan na sliki 3, je Grant s sodelavci [10] opisal kot model »jajčne škatle« (egg box model). S pomočjo elektrostatskih interakcij se povežejo nezaestrene ali nizko zaestrene galakturonske skupine z negativnim nabojem in kalcijevi ioni s pozitivnim nabojem. Kalcijev ion poveže pektin tudi z ostalimi polisaharidi. Oblikuje koordinacijsko vez med hidroksilno skupino polisaharida in ionsko vez s karboksilno skupino pektina. Odstranitev kalcijevega iona pospeši razpad pektinskih substanc, bogatih s kalcijem [11].

Dvovalentne kovinske soli pektina so v vodi netopne, topijo pa se v raztopini natrijevega hidroksida. Pri tem nastane pektinska kisli-

unesterified or slightly esterified galacturonic groups with negative charge and calcium ions with positive charge form bonds. A calcium ion also bonds pectin with other polysaccharides. It forms a coordination bond between the hydroxyl group of the polysaccharide and an ionic bond with the carboxyl group of pectin. The removal of the calcium ion enhances the decomposition of the pectin substances rich in calcium [11].

Bivalent metal salts are not soluble in water; however, they are solubilized in sodium hydroxide solutions, either by the formation of pectic acid or by an exchange between bivalent metallic ions and sodium cations. They can be also hydrolysed by suitable enzyme systems (pectinases). Compared to cellulose and hemicellulose, pectin is much more reactive [2–4, 13].

Pectin substances can be classified into four main types according to the type of modification of the main chain [7, 8]:

1. *protopectin is water insoluble pectin substance, which is the basic component of the pectin complex in plants. Upon hydrolysis pectin or pectinic acid are formed.*
2. *pectic acid is the soluble polygalacturonic acid that contains negligible amount of methoxyl groups. Normal or acid salts of pectic acid are called pectates.*
3. *pectinic acid is the polygalacturonan chain that contains less than 75 % methylated galacturonate units. Normal or acid salts of pectic acid are referred to as pectinates. Pectinic acid has the property of forming a gel with sugar or acid or, if suitably low in methyl content, with certain other compounds such as calcium salts.*
4. *pectin (polymehtyl galacturonate) is the polymeric material in which, at least, 75 % of the carboxyl groups of galacturonate units are esterified with methanol. In cell wall it may be interlined with other structural polysaccharides and proteins to form insoluble protopectin.*

3 Enzymes

Enzymes are biological catalysts that accelerate the rate of chemical reactions [2]. The reaction happens with lower activation energy which is

na ali pride do izmenjave med dvovalentnim kovinskim ionom in natrijevim kationom. Hidrolizirajo jih tudi primerni encimski sistemi (pektinaze). V primerjavi s celulozo in hemicelulozo je pektin bolj reaktiven [2–4, 13].

Pektinske substance se glede na vrsto modifikacije glavne verige delijo v štiri glavne tipe [7, 8]:

1. protopektin je vodonetopna pektinska substanca, ki je osnovna sestavina pektinskega kompleksa v rastlinah. Pri hidrolizi nastane pektin ali pektinska kislina.
2. pektinska kislina je topna poligalakturonska kislina, ki vsebuje zanemarljiv delež metoksilnih skupin. Normalne ali kisle soli pektinske kisline se imenujejo pektati.
3. pektininska kislina je molekula poligalakturonske kisline, ki vsebuje manj kot 75 % karboksilnih skupin galakturonskih enot zaestrenih z metanolom. Normalna ali kisla sol pektinske kisline se imenuje pektinat. Pektininska kislina lahko formira gel s sladkorjem in kislino, v primeru nizke koncentracije metilnih skupin pa z določenimi drugimi komponentami, kot so na primer kalcijeve soli.
4. pektin (polimetilni galakturonat) je polimerna snov, kjer je vsaj 75 % ali več karboksilnih skupin galakturonskih enot zaestrenih z metanolom. V celični steni se lahko poveže z drugimi polisaharidi in proteini, ki skupaj tvorijo netopen protopektin.

3 Encimi

Encimi so biološki katalizatorji, ki pospešujejo hitrost kemičnih reakcij [2]. Reakcija poteka z manjšo aktivacijsko energijo, kar dosežejo z oblikovanjem intermediata encim – substrat. Na substrat se vežejo s kombinacijo vodikovih in ionskih vezi ter hidrofobnimi in Van der Waalsovimi interakcijami. Kovalentne vezi nastanejo le občasno [2]. Pri sami reakciji se encimi ne porabljajo, ne postanejo del končnega produkta reakcije, temveč spreminjajo le kemične vezi drugih spojin. Vsak encim katalizira specifično reakcijo [14]. Po zaključku reakcije se sprostijo in lahko ponovno sodelujejo v naslednji biokemijski reakciji. Preprosta reakcija, kjer se substrat A preoblikuje v produkt B, reakcija pa je katalizirana z encimom E, je lahko predstavljena na naslednji način:

$$A + E \rightarrow A\text{–}E \rightarrow E + B \qquad (1)$$

Encim substrat veže in ga dogradi ali pa razcepi na manjše molekule. Vsaka vrsta encima deluje specifično in je prilagojena za točno določeno nalogo.

Encimi se med seboj ločijo glede na obliko molekule in porazdelitev naboja aktivnega mesta. Aktivno mesto je področje, kjer poteče kataliza in je le majhen del encima. Zagotoviti mora, da se substrat veže ter da ostale molekule ne motijo katalize. E. Fischer je že leta 1890 ponazoril obliko aktivnega mesta encima in komplementar-

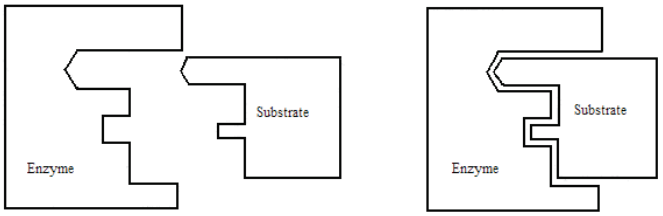
reached by forming an intermediate enzyme – substrate. Enzymes bind to substrates by a combination of hydrogen and ionic bonds, as well as by hydrophobic and Van der Waals interactions. Covalent bonds are only occasionally formed [2]. In the reaction itself the enzymes are not used up, they do not become a part of the final product of the reaction, but only change the chemical bonds of other compounds. Each enzyme catalyses a specific reaction [14]. At the end of the reaction they are released and can participate again in the next biochemical reaction. A simple reaction, where substrate A is transformed into product B and the reaction is catalysed by enzyme E, can be represented in a following way: (Equation 1).

The enzyme substrate bond and upgrades it or splits it into smaller molecules. Each type of enzyme works specific and it is adapted for precise and define reaction.

Enzymes are distinguished according to the form of the molecule and the charge distribution of the active side. The active side is an area where catalyses occurs and is just a small part of the enzyme. It must provide an environment where the substrate can bond and other molecules do not interfere with catalyses. E. Fisher, as far back as 1890, illustrated the shape of the active side of the enzyme and the complementary of the substrate and thus explained the specifics of enzyme action, which has become known as the 'lock and key' model. The active side of the enzyme, the lock, with an accurately defined rigid structure can only suit a substrate, the key, which is adapted only to it.

Enzymes differ from chemical catalysts in several important characteristics [2]:

- *enzymes catalysed reactions are several times faster than chemically catalysed ones. Compared to the non-catalysed reaction the rates is from 10^8 to 10^{10} higher [15],*
- *enzymes have far greater reaction specificity than chemically catalysed reactions and rarely form byproducts,*
- *enzymes catalyse a reaction under mild reaction conditions: the temperature is below 100 °C, the atmospheric pressure and a pH of around 7 are needed.*



Picture 4: Schematic representation of the 'lock and key' mechanism [15].

nost substrata in na ta način razložil specifičnost encimskega delovanja. To je postalo znano kot model ključavnice in ključa. Aktivno mesto encima (ključavnica) s točno definirano togo strukturo se lahko prilega le k substratu (ključ), ki je prilagojen le zanj.

Od kemičnih katalizatorjev se encimi razlikujejo v več pomembnih značilnostih [2]:

- encimsko katalizirane reakcije so nekajkrat hitrejše kot kemično katalizirane reakcije. V primerjavi z nekatalizirano reakcijo je hitrost za od 10^8 do 10^{10}-krat večja [15],
- encimi imajo veliko večjo specifičnost reakcije kot kemično katalizirane reakcije in redko tvorijo stranske produkte,
- encimi katalizirajo reakcijo pod zelo milimi reakcijskimi pogoji: temperatura je pod 100 °C, uporablja se atmosferski tlak in pH okoli 7.

3.1 Kemična zgradba encimov

Glede na kemično zgradbo encime uvrščamo med proteine. Proteine sestavlja en ali več polipeptidov, ki so sestavljeni iz zaporedja določenih aminokislin, med seboj povezanih s peptidno vezjo. V polipeptidih se pojavlja okoli 20 različnih aminokislin. Imajo sredinski (α) ogljikov atom, na katerega so vezane primarna amino skupina ($-NH_2$), karboksilna skupina (O=CR–OH ali –COOH), vodikov atom (H) in stranska polarna ali nepolarna skupina. Polarne skupine, kot so –COOH, –OH, $-NH_2$, –SH, $-CONH_2$, so ionskega karakterja, hidrofilne in vodotopne. V vodnih raztopinah so lahko stabilizirane z vodikovimi vezmi. Nahajajo se večinoma na površini encima. Nepolarne skupine (arilne in alkilne verige), ki se nahajajo v notranjosti encima, pa so relativno vodonetopne in bolj topne v organskih topilih.

Peptidna vez, ki združuje aminokisline v polipeptide, nastane z odstranitvijo vode. Je toga in planarne strukture, ostale vezi polipeptida pa lahko prosto rotirajo. Zaradi zmanjšanja steričnega vpliva med sosednjimi stranskimi verigami nastanejo intermolekulske vodikove vezi. Nastala polipeptidna veriga ima prosto eno karboksilno in eno amino skupino [2]. Edina kovalentna vez, poleg glavne poliamidne vezi, so –S–S– disulfidni mostovi. Celotna struktura je stabilizirana z velikim številom relativno šibkih Van der Waalsovih interakcij alifatske verige, π–π kompleksom aromatskih enot ali vezmi med nabitimi deli molekule.

3.1 Chemical structure of enzymes

According to their chemical structure enzymes are classified as proteins. Proteins consist of one or more polypeptides and each polypeptide is a chain of amino acids linked together by peptide bonds. 20 different amino acids could be found in polypeptides. They have a central (α) carbon atom to which is attached a primary amino group (–NH_2), a carboxyl group (O=CR–OH or –COOH), a hydrogen atom (H) and a side polar or nonpolar group or chain. Polar groups such as –COOH, –OH, –NH_2, –SH, –$CONH_2$ are ionic, hydrophilic and water soluble. They may be stabilized by hydrogen bonding in aqueous solution. They are mostly found on the surface of the enzyme. Nonpolar groups (aryl and acyl chains) that are found in the inner part of the enzyme are relatively insoluble in water, but more soluble in organic solvents.

The peptide bond that bonds the amino acids into polypeptides is formed by removing water. It is rigid and of planaric structure, whiles the other polypeptide bonds can rotate freely. Due to decrease in steric influence between the neighbouring side chains, intermolecular hydrogen bonds are formed. The formed polypeptide chain has one free carboxyl and one free amino group [2]. The only covalent bond besides the main polyamide bond is the –S–S– disulphide bridges. The whole structure is stabilised by a large number of relatively weak Van der Waals interactions of aliphatic chain, π–π complex of aromatic units or bonds between the charged parts of the molecule.

The surface of the enzyme is coated with a layer of water which is strongly bonded. The remaining water, approximatively 5 to 10 % compared to the entire weight of a dry enzyme, is referred to as structural water. It is a typical part of an enzyme that is needed to maintain the three-dimensional structure of the enzyme (and consequently its activity) and is considerably different in its physical form from the mass water in the solution that it is coated by. The rotation of the bonded water is very limited and cannot freely reorientate during freezing. A radical drying of the enzyme (in a chemical sense) forces the molecule to change its configuration and thus lose its activity.

Površina encima je prevlečena s plastjo vode, ki je močno vezana. Preostala voda, približno 5 do 10 % glede na celotno težo suhega encima, se imenuje strukturna voda. Je značilen del encima, ki je potreben, da se obdrži tridimenzionalna struktura encima (in s tem njegova aktivnost) in se v fizični obliki močno razlikuje od masne vode v raztopini, ki ga obdaja. Rotacija vezane vode je zelo omejena in se ne more prosto preorientirati med zmrzovanjem. Temeljito sušenje encima (v kemičnem smislu) sili molekulo, da spremeni konfiguracijo in tako izgubi aktivnost.

3.2 Pektinaze

Pektinolitični encimi ali pektinaze so heterogena skupina sorodnih encimov, ki hidrolizirajo pektinske substance, prisotne predvsem v rastlinah [7]. Po mednarodni nomenklaturi se glede na specifičnost reakcij uvrščajo v tretjo skupino (EC3) – hidrolaze [14]. Reakcijo katalizirajo s pomočjo vode po naslednji enačbi:

$$A–X + H_2O \rightarrow X–OH + HA \qquad (2)$$

Pektinaze proizvajajo številni mikroorganizmi, kot so naprimer bakterije in glive. Skoraj vsi komercialni proizvodi pektinaz se pridobivajo iz gliv, v industrijski proizvodnji pektinolitičnih encimov najpogosteje uporabljajo glive vrste Aspergillus niger [7]. Ta vrsta mikroorganizma ima status GRAS (Generally Regarded As Safe), kar pomeni, da so iz njih proizvedeni metaboliti varni za uporabo. Iz njih se lahko proizvajajo številne vrste pektinaz, vključno s polimetilgalakturonazami, poligalakturonazami in pektinesterazami. Proizvajajo pa jih tudi iz drugih mikroorganizmov, kot so Penicillium frequentans, Mucur pusilus in drugih. Njihova stabilnost je najboljša v območju pH med 5 in 6. S pomočjo gensko spremenjenih mikroorganizmov (predvsem različnih vrst organizmov Bacillus) so razvili tudi alkalne pektinaze, ki so stabilne v območju pH med 8 in 9. Glavni proizvajalci komercialnih produktov pektinaz so Novozymes (Nizozemska), Novartis (Švica), Roche (Nemčija) in Biocon (Indija) [16]. Produkte pektinaz lahko zasledimo pod različnimi imeni. Kisle pektinaze tržijo pod naslednjimi komercialnimi imeni: Forylase KL – Cognis, Viscozyme 120 L – Novozymes, Pectinase P9179, Pectinase p3026 – Sigma Chemical Co., Pectinase 62L – Biocatalysts, Multifect pectinase PL – Genencor International, Beisol HP – CHT; alkalne pektinaze pa pod naslednjimi komercialnimi imeni: Bioprep 3000L, Pulpzyme HC, Scourzyme L – Novozymes, Baylase EVO – Bayer, Unizim PEC – Color-Center SA, Beisol PRO – CHT, ter številnimi drugimi imeni.

3.3 Uporaba pektinaz

Pektinaze spadajo med prve namensko uporabljene encime. Pri pripravi vin in sokov so jih komercialno uporabili že daljnega leta 1930. Šele leta 1960 so kemično naravo rastlin bolj raziskali, zaradi česar je postala uporaba encimov bolj smotrna [8]. S poznavanjem strukture pektinskih substanc in mehanizma, s katerim pektinoli-

3.2 Pectinases

The pectinolitic enzymes or pectinases are a heterogeneous group of related enzymes that hydrolyse pectin substances present mainly in plants [7]. According to an international nomenclature they are classified into the third group (EC3) – hydrolases [14] due to the specifics of the reactions. The reaction is catalysed with the help of water (Equation 2).

The pectinases are produced by numerous microorganisms such as bacteria and fungi. Almost all of the commercial products of the pectinases are extracted from fungi, in industrial production of pectinolitic enzymes fungi Aspergillus niger [7] are most commonly used. This type of microorganism has a GRAS (Generally Regarded As Safe) status meaning that the produced metabolites are safe for use. Numerous types of pectinases can be produced from them including polymethylgalacturonases, polygalacturonases and pectinesterases. They can also be produced from other types of microorganisms, such as Penicillium frequentans, Mucur pusilus and others. Their stability is best in the pH range between 5 and 6. With the help of genetic changed microorganisms the alkaline pectinases were produced, they are active in the pH range between 8 and 9. The leading producers of the commercial products of pectinases are Novozymes (Netherlands), Novartis (Switzerland), Roche (Germany) and Biocon (India) [16]. Products of pectinases are found under different names. Acid pectinases are marketed under the following commercial names: Forylase KL – Cognis, Viscozyme 120 L – Novozymes, Pectinase P9179, Pectinase p3026 – Sigma Chemical Co., Pectinase 62L – Biocatalysts, Multifect pectinase PL – Genencor International, Beisol HP – CHT; and alkaline pectinases under the following commercial names: Bioprep 3000L, Pulpzyme HC, Scourzyme L – Novozymes, Baylase EVO – Bayer, Unizim PEC – Color-Center SA, Beisol PRO – CHT as well as numerous other names.

3.3 The application of pectinases

Pectinases were some of the first enzymes to be used in homes. Their commercial application was first observed in 1930 for the preparation of wines and fruit juices. Only in 1960s

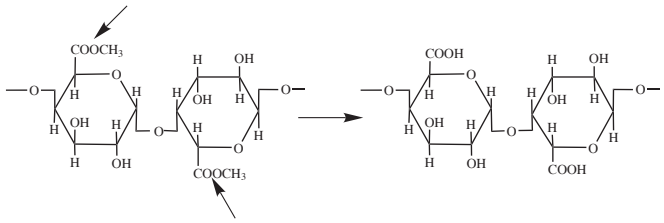
tični encimi razgrajujejo pektinske substance, se je uporaba močno povečala [16]. Tako so v današnjem času pektinaze med encimi, ki imajo najboljšo perspektivo za nadaljnjo uporabo. Pomembno vlogo predstavljajo v živilski industriji pri proizvodnji sokov, obdelavi odpadnih vod, fermentaciji kave in čaja, pripravi živalske krme in ekstrakciji citronskega olja, v papirni industriji in za druge različne biotehnološke aplikacije [7]. V tekstilni industriji uporabljajo pektinaze kot sredstvo za izkuhavanje bombaža ter pri biopripravi stebelnih vlaken, kot so lan, ramija in juta [14].

3.4 Vrste pektinaz

Pektinaze se delijo na tri skupine [8]:
- glede na vrsto substrata, ki ga razgrajujejo: pektin, pektinska kislina, oligo-D-galakturonat,
- glede na vrsto reakcije, s katero katalizirajo razgradnjo: trans-eliminacija ali hidroliza,
- glede na razcep, ki je lahko naključni (endo- tip pektinaz) ali na koncu verige (ekso- tip pektinaz).

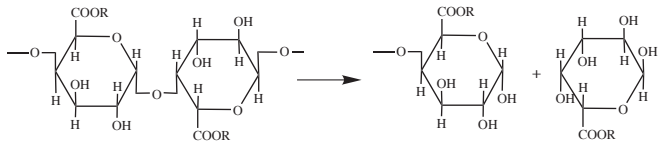
Pektinske substrate razgrajujejo naslednji tipi encimov [2, 7, 16–18]:
- pektinesteraze (EC 3.1.1.11) katalizirajo deesterifikacijo metilne skupine polimetilgalakturona, nastane pektinska kislina (pektat) in etanol:

$$pectin + nH_2O \xrightarrow{\text{pectinesteraze}} pectate + nC_2H_5OH \qquad (3)$$



Picture 5: Schematic representation of the place of attack of the pectinesterases and formation of the pectic acid [7, 16].

- poligalakturonaze katalizirajo hidrolizo 1,4-α-glikozidne vezi v poligalakturonatu z vpeljavo vode preko kisikovega mostu. Delijo se na dve skupini glede na vrsto napada polimera: endo-poligalakturonaze (EC 3.2.1.15) hidrolizirajo pektinsko kislino naključno, medtem ko eksopoligalakturonaze (EC 3.2.1.67)



Picture 6: Schematic representation of the cleavage of the polygalacturonic acid with polygalacturonases [7, 16]. R = H for the polygalacturonase (EC 3.2.1.15) and R = CH_3 for the polymethylgalacturonases.

was the chemical nature of plant tissues become apperent and with this knowledge, researcher began to use a greater range of enzymes more efficiently [8]. By learning about the structure and mechanisms of the pectin substances by which pectinolitic enzymes degrade pectin substances, their application has increased considerably [16]. Thus, pectinases are today among the enzymes with the best perspective for further application. They play an important role in the production of juices in food industry, in processing of the waste waters, the fermentation of coffee and tea, the preparation of animal forage and extraction of citric oil, in paper industry and have other biotechnological applications [7]. In the textile industry pectinases are used as agents in cotton scouring and in the biopreparation of bast fibers such as flax, ramie and jute [14].

3.4 Types of pectinases

Pectinases are classified into three groups [8]:

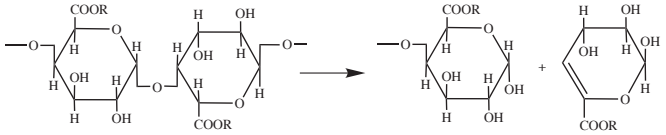
- *according to the type of substrate they degrade into: pectin, pectic acid or oligo-D-galacturonate,*
- *according to the type of reaction used to catalyse the degradation: trans-elimination or hydrolysis,*
- *according to the cleavage that can be random (endo- type of pectinases) or at the end of the chain (endwise or exo- type of pectinases).*

The pectic substrates are degraded by the following types of enzymes [2, 7, 16-18]:

- *pectinesterases (EC 3.1.1.11) catalyze deesterification of the methoxyl group of polymethylgalacturonate to release pectic acid (pectate) and ethanol (Equation 3 and Picture 5).*
- *polygalacturonases catalyse the hydrolytic cleavage of 1,4-α-glycosidic bonds of polygalacturonate with introduction of water across the oxygen bridge. They can be classified into two groups according to the type of attack of the polymer: endopolygalacturonases (EC 3.2.1.15) caused random cleavage while exopolygalacturonases (EC 3.2.1.67) caused sequential cleavage from non-reducing end of the pectin chain (Picture 6).*
- *pectin lyases cleave polygalacturonate or pectin chains via β-elimination resulting in the formation of a double bond between C4 and* cepijo na koncu verige, običajno iz nereducirajočega konca pektinske kisline.
- pektin liaze cepijo poligalakturonate ali pektinske verige preko β-eliminacije, nastane dvojna vez med četrtim in petim ogljikovim atomom na nereducirajočem koncu, CO_2 se izloči. Obstajajo trije glavni tipi liaz: endopoligalakturonat liaze (EC 4.2.2.2), ki naključno cepijo poligalakturonske verige, eksopoligalakturonat liaze (EC 4.2.2.9), ki cepijo na koncu verige poligalakturonata in nastajajo nenasičeni galakturonati ter endopolimetilgalakturonat liaze (EC 4.2.2.10), ki cepijo pektin naključno, pri čemer nastajajo nenasičeni metiloligogalakturonati. Reakcije, katalizirane z liazami, so lahko prikazane na sledeči način:

polygalacturonate $\xrightarrow{\text{poligalacturonate lyases}}$ *Δ 4 : 5 unsaturated galacturonates* (4)

polymethylgalacturonate $\xrightarrow{\text{polimethylgalacturonate lyases}}$ *unsaturated methyloligogalacturonates* (5)

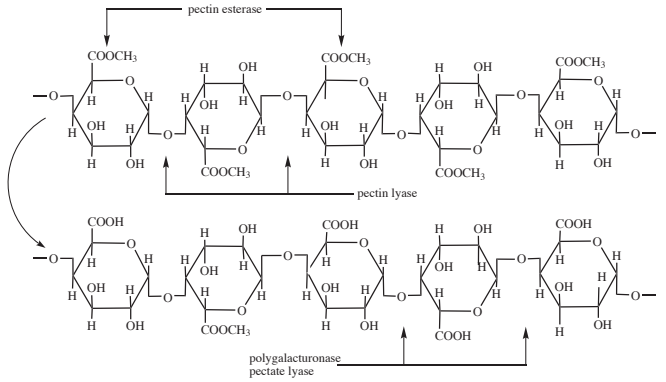


Picture 7: Schematic representation of the cleavage of pectin with pectin lyases [7, 16]. R = H for the polygalacturon lyases and R = CH_3 for the pectin lyases (EC 4.2.2.10).

- protopektinaze katalizirajo raztapljanje protopektina z naslednjo reakcijo:

protopectin (insoluble) + H_2O $\xrightarrow{\text{protopectinase}}$ *pectin (soluble)* (6)

Delijo se na osnovi reakcijskega mehanizma na A-tip protopektinaz, te reagirajo z notranjim delom, to je poligalakturonskim kislim področjem protopektina, ter na B-tip protopektinaz, te pa reagirajo z zunanjim delom, to je polisaharidnimi verigami, ki lahko povezu-



Picture 8: Illustration of action sides of different pectinases on pectin [19].

C5 at the non-reducing end, CO_2 is liberated. There are thee main types of lyases: endopolygalacturonate lyases (EC 4.2.2.2), which randomly cleave polygalacturonate chains, exopolygalacturon lyases (EC 4.2.2.9), which cleave at the chain end of polygalacturonate yielding unsaturated galacturonates and endopolymethylegalacturonate lyases (EC 4.2.2.10), which randomly cleave pectin producing non-saturated methyloligogalacturonates.

The reactions catalysed by lyases can be represented in the following way: (Equation 4, Equation 5 and Picture 7).

– *protopectinases catalyze the solubilization of protopectin with the following reaction: (Equation 6).*

They split on the basis of a reaction mechanism of A-type protopectinases which react with the inner site, i.e. the polygalacturonic acid region of protopectin, wheras B-type protopectinases react on the outer side, i.e. on the polysaccharide chains that may connect the polygalacturonic acid chain and cell wall constituents.

The mixture of all the enzymes almost entirely degrades pectin to the galacturonic acid. Picture 8 shows areas of the attack of different pectinases enzymes on pectin. Pictures 6, 7 and 8 show that the pectinases catalyze the degadation of 1,4-α-glycosidic bond in pectin causing the polygalacturonic acid to break into smaller water soluble units.

3.5 Measuring the activity of pectinases

For determining the activities of pectinases different procedures can be used, depending on the type of pectinases [7, 17, 20, 21]. Protopectinase activities are assayed by measuring the amount of pectic substance liberated from protopectin by the carbazole-sulphuric acid method. The pectin concentration is measured as D-galacturonic acid from its standard curve. One unit of polypectinase activity is defined as the enzyme that liberates pectin substance corresponding to 1 µmol D-galacturonic acid per millilitre of reaction mixture under assay conditions.

The activity of polygalacturonases can be determined by measuring the rate of increase in number of reducing groups with colorimetric methods or arsenmolybdate-copper meth-

jejo poligalakturonsko kislo verigo in konstrukcijo celične stene.
Mešanica vseh encimov skoraj v celoti razgradi pektin do galakturonske kisline. Slika 8 prikazuje mesta napada različnih pektinaznih encimov na pektin.
Iz slik 6, 7 in 8 je razvidno, da pektinaze katalizirajo razpad 1,4-α-glikozidne vezi v pektinu. Pri tem razpade poligalakturonska kislina v manjše vodotopne enote.

3.5 Merjenje aktivnosti pektinaz

Za določanje aktivnosti pektinaz se lahko uporabijo različni postopki, kar je odvisno od tipa pektinaz [7, 17, 20, 21]. Aktivnost protopektinaz se določa z merjenjem količine pektinske substance, ki se sprošča iz protopektina, s karbazol-žvepleno kislo metodo. Koncentracija pektina se določa kot sproščena D-galakturonska kislina s pomočjo umeritvene krivulje. Ena enota aktivnosti protopektinaze je definirana, ko encim sprosti 1 µmol D-galakturonske kisline na mililiter reakcijske mešanice pri preučevanih pogojih.
Aktivnost poligalakturonaz se lahko določa z merjenjem hitrosti naraščanja reducirajočih skupin s kolorimetrično metodo in arzenomolibdat-bakrovo metodo. Ena enota aktivnosti poligalakturonaz je definirana, ko encim sproti 1 µmol ml^{-1} min^{-1} galakturonske kisline pri standardnih pogojih. Uporabna je tudi metoda znižanja viskoznosti raztopine pektina. Enota encimske aktivnosti je najpogosteje izbrana kot količina potrebnega encima, ki povzroči določeno znižanje viskoznosti v enoti časa.
Najbolj primerna metoda za merjenje aktivnosti pektin liaz je merjenje porasta absorbance raztopine pektina, na katero je učinkovala znana koncentracija pektinaz pri 235 nm. Enota encimske aktivnosti je definirana kot količina encima, ki sprosti 1 µ mol nenasičenega produkta na minuto pri preučevanih pogojih. Uporabni sta tudi metodi merjenja reducirajočih skupin in redukcije viskoznosti raztopine pektina.

4 Izkuhavanje

4.1 Alkalno izkuhavanje

Najbolj razširjen postopek za odstranitev primesi iz bombaža je postopek izkuhavanja z NaOH (klasično ali alkalno izkuhavanje). Postopek poteka pri visoki temperaturi v kopeli, ki vsebuje do 4 % NaOH, različne tenzide in emulgirna sredstva. Pri tem postopku se učinkovito odstranijo voski, pektini, hemiceluloza in proteini iz kutikule ter primarne stene bombažnih vlaken. S površine vlaken se odstranijo tudi prah, različne kovinske soli, kemijske in procesne nečistoče, delno pa se odstranijo tudi nezrela vlakna in semenske luščine. Poleg naštetih prednosti pa ima postopek tudi slabosti. Na vlaknih lahko v alkalnem ob stiku z zračnim kisikom nastaja oksiceluloza. V primeru neenakomerne koncentracije raztopine je izkuhavanje neegalno, mestoma pa lahko pride celo do merceriziranja bombažnega vlakna. Po končanem postopku je potrebno

od. One unit of enzyme activity is defined as the enzyme releases 1 µmol ml^{-1} min^{-1} galacturonic acid under standard assay conditions. The decrease in viscosity of the substrate solution is a useful method as well. The unit of enzyme activity is mostly selected as the amount of enzyme required for attaining a certain decrease of viscosity per unit time.

The most convenient method of following the activity of lyases is to measure the increase in absorbance at 235 nm. One unit of enzyme activity is defined as the amount of enzyme that releases 1 µmol of unsaturated product per minute under assay conditions. Reducing group methods and viscosity reduction method are also useful in determing the lyase activity.

4 Scouring

4.1 Alkaline scouring

The most commonly used procedure for removing noncellulosic material from cotton is the procedure of scouring with NaOH (classical or alkaline scouring). The procedure is performed at a high temperature in a bath that contains up to 4 % NaOH, different surfactants and emulsifier agents. Waxes, pectins, hemicellulose and proteins from the cuticle and the primary wall of the cotton fibres are efficiently removed in this procedure. Dust, different metal salts, chemical and processing impurities are also removed from the surface of the fibres, and partly also immature fibres and seed husks. Besides all the advantages mentioned, the procedure has some disadvantages. In the alkaline medium in contact with oxygen from the air oxycellulose can be formed on fibres. In cases when the solution is irregular, the scouring is unequal since mercerisation of the cotton fibre can occur randomly. Having concluded the procedure, the fabric needs to be thoroughly rinsed and neutralised, with a considerable amount of water used in the process. Salts formed in neutralisation needs special procedures of cleaning. Due to high temperature a lot of energy is consumed in the process.

The efficiency of scouring is evaluated by determining residues of the different types of impurities, especially waxes and pectin that are found on the fibres. Cracks are formed on the fibres,

tkanino temeljito izpirati in nevtralizirati, za kar se porabi velika količina vode. Pri nevtralizaciji nastajajo tudi soli, ki potrebujejo specialne postopke čiščenja. Zaradi visoke temperature se porabi tudi velika količina energije.

Učinkovitost izkuhavanja se ovrednoti z določevanjem preostanka različnih tipov nečistoč, predvsem voskov in pektina, ki se nahajajo na vlaknih. Na vlaknih nastanejo razpoke, kutikula in primarna stena se odstranita. Fina struktura vlakna se ne spremeni, le malenkostno se spremeni stopnja kristaliničnosti bombaža. Največja sprememba na bombažni tkanini je izguba mase. Dolžina tkanine se zaradi skrčenja med vrenjem skrajša, zato se spremenita gostota in pretržna sila, ki se običajno povečata. Najpomembnejša sprememba pa je povečana omočljivost, ki je nujna lastnost za dobro in enakomerno beljenje, barvanje in končne obdelave. Omočljivost mora biti dobra ne samo v prostorih med vlakni, temveč tudi v notranjosti vlakna [13].

4.2 Bioizkuhavanje

Bioizkuhavanje je postopek izkuhavanja bombaža, kjer se namesto alkalije uporablja ekološko sprejemljivejše encime pektinaze. Postopek poteka pri temperaturi do 60 °C, maksimalno eno uro. Glede na tip pektinaz je kopel rahlo kisla ali rahlo alkalna. V kopel je priporočljivo dodajati neionski tenzid in, odvisno od tipa pektinaz, kompleksant. Postopek je osnovan na dejstvu, da pektin deluje kot vrsta cementa ali lepila, ki stabilizira primarno steno bombažnih vlaken. Pri delovanju pektinaz nastane med pektinazo in pektinom kompleks, kar povzroči hidrolizo pektinskih substanc. Rezultat te delne hidrolize je razcep vezi med kutikulo in celuloznim telesom [22]. Zunanje plasti se destabilizirajo in se v sledečih postopkih izpiranja odstranijo. Encimi se sprostijo in se ponovno povežejo s pektinom. Postopek se ponavlja, dokler encim ne uničimo kemično, s spremembo pH ali temperature [23]. Z odstranitvijo pektina, ki v vlaknih deluje kot lepilo med celuloznim in neceluloznim delom, pa se odstranijo tudi ostale necelulozne snovi. Postopek bioizkuhavanja da na otip mehkejša vlakna kot konvencionalno izkuhavanje, vendar je stopnja beline slabša in postopek ni primeren za odstranjevanje semenskih primesi in nopkov [2]. Potencialne prednosti, ki delajo encimatsko izkuhavanje komercialno privlačno, vključujejo boljšo kakovost vlaken (boljši otip in višjo trdnost), manj odpadne vode, prihranke energije in kompatibilnost z drugimi postopki, opremo in materiali [12].

4.3 Razvoj in pogoji bioizkuhavanja

Začetne študije encimskih obdelav za izkuhavanje oziroma čiščenje bombažnih vlaken, ki so jih izvajali nemški raziskovalci [24-27], so vključevale pektinaze, proteaze in lipaze, ki delujejo na bombažne nečistoče, ter celulaze, ki hidrolizirajo celulozno verigo. Sledili so številni drugi raziskovalci: Li, Hardin, Hartzell, Buchert, Traore in Karapinar s sodelavci [22, 19, 28–31]. Ugotovili so, da so najučinkovitejše celulaze in pektinaze, manj lipaze in najmanj pro-

cuticule and the primary cell are removed. The fine structure of the fibre does not change, only the degree of crystallinity of cotton is slightly changed. The most noticeable change in the cotton fabric is the loss of mass. The lenght of the fabric shortens during boiling due to shrinking, causing the increasing of density and the tearing force. The most important change is the increased wettability which is a necessary property for a successful and even bleaching, dyeing and final treatment. The wettability needs to be good not only in spaces between the fibres but in the inner parts of the fibre as well [13].

4.2 Bioscouring

Bioscouring is a procedure of scouring of cotton where ecologically more acceptable pectinases enzymes are used instead of alkali. The procedure is in progress for one hour maximum at a temperature of 60 °C. Considering the type of pectinases the bath may be slightly acidic or alkaline. It is recommendable to add the non-ionic surfactant into the bath and, depending on the type of the pectinases, a sequestering agent. The procedure is based on a fact that pectin acts as a type of cement or glue that stabilises the primary cell of the cotton fibres. When the pectinases are active a complex is formed between the pectinases and the pectin which causes the hydrolysis of the pectin substances. The result of this hydrolysis is a split of the bond between the cuticle and the cellulose body [22]. The outer layers are destabilised and removed in the following procedures of rinsing. The enzymes are released and bond again with the pectin. The procedure is repeated until the enzyme is not destroyed chemically, with the change in pH or in the temperature [23]. By removing pectin, which acts as a glue between the cellulose and noncellulose part, other noncellulose substances are removed. The procedure of bioscouring gives softer fibres than conventional scouring, however the degree of whiteness is lower and the procedure is not appropriate for removing seed-coat fragments [2]. The potential advantages that make the enzyme scouring commercially appealing, are a higher quality of the fibres (softer to the touch and better strength), less waste waters, economy of energy and compatibility with other procedures, equipment and materials [12].

teaze. Na osnovi svojih študij so zaključili, da preprost postopek s pektinazami v prisotnosti neionskega tenzida zadošča za doseganje dobre vpojnosti [23, 30, 32–41]. Izkuhavanje s pomočjo celulaz se kljub dobri vpojnosti materiala ni razširilo, ker je na vlaknih prišlo do prevelikih poškodb [29].

Prve raziskave s pektinazami kot sredstvom za izkuhavanje bombaža so bile narejene za optimiziranje pogojev njihovega delovanja. Na aktivnost encimov vpliva koncentracija encimov v kopeli, čas in temperatura obdelave, pH kopeli, dodatki v kopel in mehansko delovanje.

Koncentracija pektinaz

Zaradi zelo različnih encimskih pripravkov se dodana količina pektinaz od raziskave do raziskave močno razlikuje. Koncentracije so običajno nizke, od 0,05 do 2 % glede na maso vlaken. Povečanje koncentracije nad predpisano (optimalno) vrednost ne pospeši niti ne izboljša učinkovitosti obdelave.

Temperatura

Temperatura bioizkuhavanja je v primerjavi s klasičnim izkuhavanjem veliko nižja, saj je optimalna temperatura od 40 do 60 °C [22, 36, 37], nad to temperaturo pektinaze izgubijo svojo aktivnost, ker višja temperatura encime uniči. Vendar pa tako nizka temperatura ne zadošča za odstranjevanje voskov, ki imajo točko tališča nad 70 °C. Za boljšo odstranitev primesi se zato najpogosteje priporoča dvig temperature kopeli po končanem izkuhavanju. Drug razlog dviga temperature je tudi deaktiviranje encimov. Same pektinaze sicer do celuloznih vlaken niso škodljive, vendar pogosto encimski preparati vsebujejo tudi sledi celulaz, ki pa bi na vlaknih lahko povzročile poškodbe. Hartzell in Hsieh [28] sta za boljšo odstranitev hidrofobnih primesi pred bioizkuhavanjem izvedli 3-krat po 2-minutno predobdelavo v vodi pri 100 °C. Ta predobdelava je imela podobne pozitivne učinke na bioizkuhavanje s pektinazo kot dodatek celulaz v izkuhalno kopel. Lenting [42] pa je za boljšo odstranitev hidrofobnih snovi predlagal višjo temperaturo izpiralne kopeli. Pri tem se hkrati s hidrofobnimi snovmi učinkovito odstranijo tudi razgrajeni ostanki pektina.

pH kopeli

Poleg temperature je ključnega pomena za aktivnost in stabilnost encima tudi pH okolja. Večina encimov je aktivna v območju pH med 5 in 9. Aktivni so v širšem območju pH, pri ekstremni vrednosti pa se tridimenzionalna oblika encimov poruši in encimi izgubijo svoje katalitično obnašanje. Alkalno ali kislo okolje je odvisno od tipa pektinaz [35, 37, 43]. Poznane so namreč kisle pektinaze, ki delujejo v rahlo kislem mediju (pH med 4 in 6), in alkalne pektinaze, ki delujejo v rahlo alkalnem mediju (pH med 7 in 9), na bombaž pa podobno učinkujeta obe vrsti pektinaz [44, 45]. V kislem pride do razpada pektinske strukture že brez dodatka pektinaz, kar je pogosto razlog boljšega delovanja kislih pektinaz v primerjavi z alkalnimi pektinazami [46].

4.3 The development and conditions of bioscouring

The starting studies of enzyme treatment for scouring that is, cleaning of cotton fibres, were carried out by German researchers [24–27], and they included pectinases, proteases and lipases that act upon impurities and cellulases which hydrolyse the cellulose chain. Many other researchers followed in their path: Li, Hardin, Hartzell, Buchert, Traore and Karapinar with colleges [22, 19, 28–31]. They established that cellulases and pectinases are the most effective ones, lipases less with proteases being the least effective. On the basis of their studies they concluded that a simple procedure with pectinases in presence of non-ionic surfactant is sufficient to attain good absorbency [23, 30, 32–41]. Scouring by cellulases has not widened in spite of good absorbency of the material since the damage to the fibres was too noticeable [29].

The first researches including pectinases as agents for scouring cotton were carried out to optimise the conditions of their activity. The concentration of the enzymes in the bath as well as time and temperature of treatment, pH of the bath, additives in the bath and the mechanical treatment all influence on the activity of the enzymes.

Concentration of pectinases

Due to a wide variety of enzyme solutions, the added amount of pectinases strongly differs from research to research. The concentrations are usually low, from 0.05 to 2 % according to the weight of the fibres. The increase of concentration above the optimal value neither enhances nor improves the efficacy of the treatment.

Temperature

The temperature of bioscouring is much lower compared to classic scouring, the optimal temperature is from 40 to 60 °C [22, 36, 37]. Above the mentioned temperature the pectinases lose their activity since a higher temperature destroys the enzymes. However, a temperature that is too low does not suffice for removing the waxes, which have a melting point above 70 °C. A raise in temperature of the bath after completing the scouring is recommended for a better removal of the noncelullulosic ma-

Čas delovanja

V začetnih raziskavah so dolge čase obdelave izpostavljali kot glavno slabost encimskega izkuhavanja [41]. Z razvojem novejših pektinaz pa so se skrajševali tudi časi obdelav. Tako sedanje oblike pektinaz potrebujejo za svoje delovanje od 30 do 60 minut [35, 47]. Lenting in Zwier [48] celo trdita, da je v kombinaciji z učinkovitim izpiranjem zadosten čas obdelave že nekaj minut.

Dodatki v kopel

Velik vpliv na odstranjevanje neceluloznih nečistoč imajo tudi dodani tenzidi, vendar je pri dodajanju tenzida v kopel potrebna zajljivost. Anionski tenzidi lahko tvorijo komplekse s proteini in vplivajo na strukturo. Kationski tenzidi podobno vplivajo na proteine, vendar z manjšo afiniteto. Katalitično aktivnost encimi običajno zadržijo v raztopini z neionskimi tenzidi (neionski tenzidi se s proteini ne povežejo), razen če koncentracija tenzida v raztopini v veliki meri preseže kritično micelno koncentracijo [40]. Neionski tenzidi so kompatibilni z encimi in ne rušijo njihove tridimenzionalne strukture. Pospešijo učinke izkuhavanja zaradi zmanjšanja površinske napetosti vlaken in lažje penetracije encima v mikropore in razpoke vlaken. Nenazadnje tenzid povleče encim nazaj v kopel, kjer je ponovno na razpolago za nadaljnje katalitično delovanje [39, 40, 43, 44]. Tenzidi sodelujejo tudi pri odstranitvi voskov in maščob [44, 49].

Topnost encimov poveča nizka koncentracija soli. Pri velikih koncentracijah soli pride do velikega števila interakcij med soljo in vodo, kar povzroči zmanjšanje interakcij med proteinom in vodo, posledica pa je izločanje encima iz raztopine [2]. Izogibati se moramo encimskih inhibitorjev, kot so težke kovine in ionski detergenti, ter produktov na osnovi formaldehida, ker deaktivirajo encim [2, 40].

Ena izmed možnosti za izboljšanje degradacije pektina je tudi dodatek kompleksanta. Znano je, da imajo kalcijevi ioni pomembno vlogo v strukturi pektina, ioni Ca^{2+} povezujejo nezaestrene molekule pektina. Z odstranitvijo tega iona se struktura pektina destabilizira, kar omogoča pektinazam lažji dostop do mest napada. Csiszar s sodelavci [50] je preučevala vpliv EDTA na delovanje pektinaz. Pri pH 5 je neaktiven, ker je pri tej vrednosti pH manj disociiran in je koncentracija kompleksov, ki nastanejo med kompleksantom in kalcijevim ionom, nizka. V nevtralnem mediju, še bolj pa v rahlo alkalnem mediju, ta kompleksant veže kalcijeve ione v večji meri, kar povzroči boljši razcep kompleksov pektina. Pri višji vrednosti pH je namreč moč kompleksiranja EDTA večja. Boljše delovanje EDTA v alkalnem ter zato boljšo razgradnjo pektinskih substanc sta potrdila tudi Gamble [51] in Preša [46]. Losonczi s sodelavci [11] je preučevala dvostopenjsko obdelavo bombaža: najprej je obdelala bombaž v raztopini kompleksanta (EDTA), ki ji je sledila obdelava z encimi pektinazami. Ugotovila je, da se pri predobdelavi kalcijevi ioni odstranijo in da se struktura pektina poruši, vendar pa se tako odprta struktura proti koncu pred-

terial. A second reason for raising the temperature is also the deactivating of the enzymes. The pectinases alone are not harmful to the cellulose fibres, however, enzyme preparations often contain traces of cellulases which could be damaging to the fibres. Hartzell and Hsieh [28] have carried out a two-minute pre-treatment in water at 100 °C three times to allow a better removal of hydrophobic alloy. The mentioned pre-treatment had similar positive effects on bioscouring with pectinases as an additive of cellulases into the scouring bath. Lenting [42] suggested a higher temperature of the rinsing bath for a better removal of hydrophobic substances. The degraded residue of pectin is successfully removed with the hydrophobic substances in the procedure itself.

pH of the baths

Beside the temperature, the pH of the environment is crucial for the activity and stability of the enzyme. The majority of enzymes are active in the pH range between 5 and 9. They are active in a wider pH range, however, at extreme values the three-dimensional form of the enzymes collapses and the enzymes lose their catalytic behaviour. Alkaline or acidic environment depends on the type of pectinases [35, 37, 43]. Acidic pectinases that function in a slightly acidic medium (pH between 4 and 6), as well as alkaline pectinases that function in a slightly alkaline medium (pH between 7 and 9) are known, both types have similar effects on cotton [44, 45]. In acidic medium the pectin structure degrades without adding the pectinases which is often the reason for a better functioning of the acidic pectinases over the alkaline pectinases [46].

Time of functioning

In the starting researches, longer times of treatment were pointed out as the main disadvantage of the enzyme scouring [41]. By developing new pectinases, the times of treatment have shortened. Thus, the present forms of pectinases need 30 to 60 minutes for their functioning [35, 47]. Lenting and Zwier [48] also claim that a few minutes of treatment are enough in combination with an effective rinsing.

obdelave sesede in ponovno zapre, kar povzroči, da je površina za napad encimov še bolj nedostopna. Pri istočasnem delovanju encima in EDTA so ugotovili, da kompleksant encimu pomaga pri razgradnji pektina. Encimi hidrolizirajo naravne polimere v substratu. Pri tem zrahljajo strukturo, kar povzroči, da je kovinski ion bolj dostopen in ga EDTA zato lažje kompleksira. EDTA izboljša učinkovitost komercialnih ksilanaz in kislih pektinaz in pospeši odstranitev nečistoč iz bombažne tkanine in degradacijo semenskih ostankov. Uporaba EDTA v raztopini encima prav tako znatno vpliva na učinkovitost sledečih kemičnih postopkov [11, 52].
Kljub dobrim rezultatom pri istočasnem delovanju kompleksanta in pektinaz, je pri izbiri kompleksanta potrebna pazljivost. Premočni kompleksanti vežejo tudi kovinski ion, ki je prisoten v določenih vrstah encimov, t. i. metalo encimih (npr. Bioprep 3000L). Odstranitev tega kovinskega iona s kompleksantom poruši strukturo encima, kar povzroči deaktivacijo encima [11, 18, 42, 46, 49]. Zato priporočajo uporabo šibkejših kompleksantov, kot so fosfatni, silikatni ali karbonatni kompleksanti [49].

Gibanje kopeli

Pri bioizkuhavanju bombaža pektinaze prodirajo v vlakna skozi kutikulo na mestih, kjer so razpoke in mikropore, in katalizirajo reakcijo hidrolize pektinskih molekul. Mešanje zrahlja vezi med primarno in sekundarno steno bombaža, kar povzroči, da na površini vlaken nastane več mikropor in razpok [40]. Zaradi tega encim lažje prodira v notranjost vlaken. Ko je biološko lepilo odstranjeno, se ostale necelulozne snovi primarne stene sprostijo in raztopijo, dispergirajo ali emulgirajo s tenzidi in šibkimi kompleksanti v bioizkuhalni kopeli. Hartzell-Lawson in Durant [47] trdita, da vpeljava mešanja v postopek izkuhavanja s pektinazami močno poveča vpojnost bombažne tkanine. Mešanje, v obliki mešanja kopeli s tkanino ali kot trenje tkanine s tkanino, optimizira pektinazni postopek. Skrajša se čas obdelave in zmanjša se količina potrebnih pektinaz za doseganje dobre vpojnosti tkanine.
Izmed naštetih dejavnikov je stopnja mehaničnega dela najmanj pomemben dejavnik [30], vendar nekoliko izboljša učinke encimov pri izkuhavanju [36, 40].

5 Vpliv bioizkuhavanja na nadaljnje postopke plemenitenja

Po bioizkuhavanju so bombažna vlakna temnejša kot po alkalnem izkuhavanju. Pogosto so bioizkuhana vlakna celo za nekaj enot temnejša kot surova, neizkuhana vlakna [46]. Pri nadaljnjem beljenju z vodikovim peroksidom so ugotovili, da se po beljenju z vodikovim peroksidom boljša stopnja beline doseže na alkalno izkuhanem vzorcu kot na bioizkuhanem vzorcu, vendar je potrebno upoštevati, da so alkalno izkuhana vlakna zelo občutljiva za oksi-

Additives in the bath

Added surfactants also have a big influence on removing noncellulosic impurities, however, caution is advised when adding surfactant. Anionic surfactants can form complexes with proteins and influence the structure. Cationic surfactants have a similar influence on proteins, however, with a lower affinity. Enzymes usually retain their catalytic activity in a solution with non-ionic surfactants (non-ionic surfactants do not bond with proteins), unless the concentration of the surfactants in the solution exceeds the critical micelle concentration [40]. Non-ionic surfactants are compatible with enzymes and do not break their three-dimensional structure. They accelerate the effects of scouring due to lowering the surface tension of the fibres and an easier penetration of the enzyme into micropores and cracks of the fibres. Ultimately, the surfactants pull the enzyme back into the bath where it is available for further catalytic activity [39, 40, 43, 44]. Surfactants take an active part in removing waxes and grease [44, 49].

The solubility of enzymes increases with a lower concentration of the salts. At high concentrations of salts a great number of interactions between the salt and water occur, which causes a decrease in interactions between the protein and water, the consequence being the elimination of the enzyme from the solution [2]. Enzyme inhibitors such as heavy metals and ionic detergents as well as product on the basis of formaldehyde need to be avoided since they deactivate the enzyme [2, 40].

One of the possibilities of improving the degradation of pectin is also the addition of the chelating agent. It is well known that calcium ions play an important part in the structure of the pectin, the Ca^{2+} ions bond the nonestrificated molecules of pectin. By removing the ion, the structure of the pectin is destabilised which enables the pectinases an easier access to the areas of attack. Csiszar and colleges [50] studied the influence of EDTA on the functioning of the pectinases. At pH 5 it is inactive, since it is less dissociated at this lower value of the pH and the concentration of the complexing species that occur between the chelating agent and the calcium ion is low as well. In a neutral medium, or even more in a slightly alkaline medium, this

dativne poškodbe med beljenjem. Pride do večjih poškodb kot na vzorcih, izkuhanih s pektinazami [53, 54]. Losonczi s sodelavci [39] in Forte s sodelavci [55] trdijo, da se bioizkuhana vlakna lažje belijo, saj je razlika v stopnji beline pred in po beljenju bioizkuhanih vlaken večja kot razlika v stopnji beline alkalno izkuhanih vlaken pred in po beljenju.

Nekaj raziskovalcev je preučevalo možnosti kombiniranja bioizkuhavanja s predhodnim ali naslednjim postopkom. Zadostno omočljivost so dosegli s kombinacijo encimskega razškrobljenja in bioizkuhavanja [42, 45]. Tzanov s sodelavci [44] je za izkuhavanje uporabil razškrobilno kopel, ki je bila v sledečem belilnem postopku z glukoza oksidazami pomemben vir glukoze. Glukoza oksidaze v vodni raztopini ob prisotnosti glukoze proizvajajo vodikov peroksid iz v vodi raztopljenega kisika. Stopnja beline dosežena pri tem postopku je nižja od stopnje beline vlaken beljenih po klasičnem postopku z vodikovim peroksidom.

Pektinaze, kisle in alkalne, se lahko v isti kopeli kombinirajo tudi s peroksiocetno kislino. Pri tem postopku istočasno poteka izkuhavanje in beljenje bombaža. Pektinaze in peroksiocetna kislina delujeta pri podobnih delovnih pogojih (temperatura 50–60 °C, času 40–60 minut, pH 5–8). Zaradi razmeroma nizke delovne temperature nastanejo prihranki pri porabi energije. Prihranki pa nastanejo tudi pri porabi vode in časa, saj se izkuhavanje in beljenje vršita v isti kopeli istočasno. Vpojnost tako obdelanih vlaken je zadostna za sledeče postopke, primerljiva s klasično peroksidno beljenimi vzorci pa je tudi stopnja beline [46, 55–59].

Obarvanje z direktnimi in reaktivnimi barvili je bilo učinkovito in egalno na različno izkuhanih tkaninah [23, 30, 33, 39, 46, 54, 60]. Etters [61] ni opazil nobene statistično značilne razlike med navzemanjem barvila, ravnotežjem izčrpanja in globino barvnega tona med tkaninami, ki so bile alkalno izkuhane ali bioizkuhane. Možen razlog dobrega navzemanja je, da tudi nizka koncentracija alkalije v barvalni kopeli spremeni morfologijo bombaža, kar poveča substantivnost nekaterih barvil. Nasprotno pa Losonczi s sodelavci [39] trdi, da se klasično izkuhana tkanina obarva svetleje kot bioizkuhana. Po predhodnem beljenju različno izkuhanih tkanin pa tudi pri svetlih obarvanjih ni opaziti razlik. Obdelave z in brez encima ne vplivajo na enakomernost obarvanja.

6 Zaključek

Bioizkuhavanje ima kar nekaj prednosti zaradi katerih ga lahko priporočimo kot primeren postopek za izkuhavanje [32, 38, 62, 63, 64]. Je enostaven, ponovljiv in varen postopek. Odstranitev pektinskih komponent iz bombaža v zadostni meri izboljša hidrofilnost vlaken in olajša penetracijo barvila ter drugih sredstev v vlakno. Naravne lastnosti bombažnega vlakna se ohranijo, otip je mehkejši kot po klasičnem izkuhavanju. Vlakna so manj poškodovana. Bioizkuhavanje lahko uporabljamo tudi za mešanice bombaža s svilo,

chelating agent bonds calcium ions more strongly which enhances a better split of the pectin complexes. At higher pH values the strength of EDTA complexing is higher. A better activity of the EDTA in the alkaline and therefore a better degradation of the pectin substances were certified by Gamble [51] and Preša [46]. Losonczi and colleges [11] studied a two-way treatment of cotton. First, she treated cotton in a chelating agent solution (EDTA) followed by a treatment with the enzymes pectinases. She discovered that in pretreatment the calcium ions are removed and the structure of pectin destroyed, however, such open structure collapses and closes again towards the end of the pretreatment, which makes the surface of the enzymes even more inaccessible for attack. In simultaneous activity of the enzyme and the EDTA the chelating agent helps the enzyme in pectin degradation. Enzymes hydrolyse the natural polymers in the substrate while loosening the structure causing the metal ion to be more accessible so EDTA can complex it more easily. ETDA improves the efficacy of commercial xylanases and acidic pectinases and accelerates the removal of impurities from the cotton fabric as well as the degradation of the seed-coat fragments. The use of EDTA in a solution can considerably influence the efficacy of the following chemical procedures [11, 52].

Despite the good results in simultaneous activity of the chelating agent and the pectinases, caution is advisable in choosing the chelating agent. Chelating agents that are too strong also bond the metal ion, which is present in some types of enzymes, the so called metalo enzymes, for example Bioprep 3000L. The removal of this metal ion with the chelating agent destroys the structure of the enzyme which causes a deactivation of the enzyme [11, 18, 42, 46, 49]. Therefore, the use of weaker chelating agents, such as phosphate, silicate and carbon chelating agents [49], is recommended.

Movement of the bath

In bioscouring, the cotton pectinases penetrate into the fibres through the cuticle in places where there are cracks and micropores, and catalyse the reaction of hydrolysis of the pectin molecules. The mixing loosens the bonds be-

volno ali kašmirjem; pri močno alkalnih pogojih klasičnega izkuhavanja namreč na teh vlaknih pride do poškodb.

Iz tekstilnih obdelovalnih postopkov se odstrani natrijev hidroksid ali se njegova poraba močno zmanjša. Zaradi nižje pH kopeli je potrebno manj izpiranja, kar ima za posledico krajše čase obdelav in manjšo porabo vode. Prihranki nastanejo tudi pri porabi energije, saj bioizkuhavanje poteka pri nižji temperaturi. V primeru barvanja temnih odtenkov je možno direktno barvanje brez vmesnega beljenja.

Odpadne vode so manj onesnažene, nižje so tako vrednosti KPK izkuhalnih kopeli zaradi manjše porabe kemikalij kot tudi vrednosti BPK zaradi manjše izgube mase vlaken.

Vendar pa ima bioizkuhavanje tudi nekaj slabosti. Zaradi razmeroma nizke temperature obdelave se voski ne odstranijo v celoti. Dosežena stopnja beline je nižja kot na alkalno izkuhani in celo na razškrobljeni tkanini. Zaradi nizkega pH semenski ostanki ne nabreknejo in se pri beljenju težje razbarvajo. Pri skladiščenju encimov je potrebno paziti, da so posode dobro zaprte, da ne vstopi voda. Za podaljšanje življenjske dobe encimov je potrebno encime skladiščiti v hladnem prostoru.

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tween the primary and the secondary wall of the cotton causing more micropores and cracks to appear on the surface of the fibres [40]. This enables the enzyme to penetrate more easily into the inner part of the fibres. When the biological glue is removed, other noncellulose substances of the primary wall are freed and they dissolve, disperse in a different substance and emulgation in a different liquid with surfactants and weak chelating agents in a bioscouring bath. Hartzell-Lawson and Durant [47] claim that introducing agitation into the procedure of scouring with the pectinases strongly enhances the absorption of the cotton fabric. Agitation, in the form of agitation bath with the fabric or as fabric-to-fabric agitation, optimises the pectinase procedure. The time of treatment is shortened and the amount of pectinases needed to attain good absorption of the fabric is lowered. Among the mentioned factors, the degree of mechanic work is the least important factor [30], however, it somehow improves the effects of enzymes in scouring [36, 40].

5 Influence of bioscouring on further finishing procedures

After the bioscouring the cotton fibres are darker than after alkaline scouring. Bioscoured fibres are often several units darker than raw, unscoured fibres [46]. In further bleaching with hydrogen peroxide, it was established that a better degree of whiteness can be attained on alkaline scoured sample than on the bioscoured one, however, it need to be taken into account that alkaline scoured fibres are very sensitive to oxidative damage during bleaching. More significant damage occures compare to the samples scoured with pectinases [53, 54]. Losonczi and colleges [39] and Forte and colleges [55] claim that bioscoured fibres are bleached more easily since the difference in the degree of whiteness before and after the bleaching of bioscoured fibres is bigger than the difference in the degree of whiteness of the alkaline scoured fibres before and after the bleaching.

Several researchers examined the possibilities of combining bioscouring with previous and following procedure. They achieved an adequate wettability by combining enzymes desizing and

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bioscouring [42, 45]. Tzanov and collegues [44] used a desizing bath for scouring and it proved to be an important source of glucose in the following procedure with the glucose oxydases. The glucose oxydases produce hydrogen peroxide in water solutions in the presence of glucose from oxygen dissolved in water. The degree of whiteness attained in this procedure is lower than the degree of whiteness of the fibres bleached in a classic procedure with hydrogen peroxide.

Pectinases, acidic as well as alkaline, can also be combined with peracetic acid in the same bath. Pectinases and peracetic acid function at similar working conditions (temperature 50–60 °C, time 40–60 minutes, pH 5–8). Due to relatively low working temperature, energy is economised. The quantity of water and time are also economised since scouring and bleaching occur simultaneously in the same bath. The absorption of fibres thus treated is sufficient for the following procedures, the degree of whiteness is also comparable with classically peroxide bleached samples [46, 55–59].

Dyeing with direct and reactive dyes was efficient and equal on fabrics that were differently scoured [23, 30, 33, 39, 46, 54, 60]. Etters [61] did not notice any statistically significant difference between the rate of uptake, equilibrium exhaustion, or colour depth on the cotton substrate between the two fabrics that were either alkaline scoured or bioscoured. A possible reason for good absorption is that even a low concentration of alkali in the dyeing bath changes in morphology of the cotton which increases the substantivity of some dyes. On the contrary Losonczi and colleges [39] claim that classically scoured fabric compared to bioscoured fabric has a lighter colour. After previous bleaching of differently scoured fabric, no differences can be noticed in lighter dyeing. Treatments with or without the enzyme do not affect the evenness of the dyeing.

6 Conclusion

Due to several advantages of bioscouring, it can be recommended as an adequate procedure for scouring [32, 38, 62, 63, 64]. It is a simple, repeatable and safe procedure. The removal of pectin components from cotton adequately im-

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proves the water absorbencies of the fibres and facilitates the penetration of the dye and other substances into the fibre. Natural qualities of the cotton fibre are preserved, the fabric is softer to the touch than after classic scouring. Fibres are also less damaged. Bioscouring can also be used for mixtures of cotton and silk, wool and cashmere; in severe alkaline conditions of classic scouring, damage occurs on these fibres.

Sodium hydroxide is removed from the textile treatment procedures or its use is considerably lowered. Due to a lower pH of the bath, less rinsing is needed, what results in shorter times of treatment and lower use of water. Energy is economised as well, since the bioscouring occurs at a lower temperature. Direct dyeing without the intermediary bleaching is possible in the case of dyeing dark shades.

Waste waters are less polluted, the KPK values of the scouring baths are thus lower due to the economised use of chemicals as well as BPK values due to a smaller loss of the fibre weight.

However, bioscouring has a few disadvantages. Due to a relatively low treatment temperature, the waxes are not entirely removed. The attained degree of whiteness is lower compared to alkaline scoured or even desized fabric. Due to a lower pH the seed-coat fragments do not swell and are not so decolorized in bleaching. In storing the enzymes, one needs to make sure the containers are well closed preventing the water to enter. Enzymes need to be stored in a cool place if longer life period is to be attained.

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