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Uporaba ATR IR spektroskopije pri proučevanju strukturnih sprememb celuloznih vlaken

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The use of ATR IR spectroscopy in the study of structural changes of the cellulose fibres

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Abstract

In the present study the application of an ATR IR spectroscopic technique on the structural changes of the cellulose fibres was studied. Two examples of the cellulose modification were studied, i.e. the finishing of cotton fibres with a novel silane precursor and the biodegradation of cotton fibres with microorganisms. It was demonstrated that the analysis of the ATR IR spectra is an appropriate method for determining the chemical properties of the thin (< 100 nm) nanocomposite film, as well as for determining its structural changes after repeated washing of finished fibres. We also showed that the ATR IR spectroscopy is appropriate for investigating the biodegradation study of cellulose fibres, as interred from the changes of crystallinity and amorphous regions of the fibres, hydrolytic and oxidative degradation of the cellulose, as well as microbial growth on the fibres.

Key words: cellulose, ATR IR spectroscopy, chemical finishing, sol-gel technology, biodegradation.

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Izvleček

Namen raziskave je bil proučiti uporabnost ATR IR spektroskopije pri študiju strukturnih sprememb celuloznih vlaken. Izbrali smo dva primera preoblikovanja, in sicer postopek sol-gel kemijske apreture vlaken z novim sredstvom na podlagi silana ter proces biorazgradnje vlaken pod vplivom mikroorganizmov. Analiza spektrov je pokazala, da se lahko z ATR IR spektroskopijo podrobno določijo kemijske lastnosti nanokompozitne apreturne prevleke kljub njeni izredno majhni debelini (< 100 nm), prav tako pa se razkrijejo strukturne spremembe nanokompozitne prevleke oplemenitenih vlaken po večkratnem pranju. ATR IR spektroskopija je prav tako primerna za spremljanje biorazgradnje celuloznih vlaken, pri čemer smo določili spremembe v kristalinosti oziroma amorfnosti vlaken v odvisnosti od časa biorazgradnje, hidrolitične in oksidacijske poškodbe celuloze in stopnjo rasti mikroorganizmov na vlaknih.

Ključne besede: celuloza, ATR IR spektroskopija, kemijska apretura, sol-gel tehnologija, biorazgradnja.

1 Uvod

Infrardeča (IR) spektroskopija je ena najpomembnejših in hkrati najpogosteje uporabljenih analitskih metod, s katero lahko razložimo kemijsko strukturo snovi in določimo njene funkcionalne skupine. Med različnimi IR tehnikami je za študij ploskovnih tekstilij posebno primerna metoda z oslabljenim odbojem (ATR IR). Slednja omogoča zaradi tesnega stika med preiskovanim substratom, kot so ploskovne tekstilije in ATR kristalom (diamant ali ger-

1 Introduction

Infrared (IR) spectroscopy is one of the most important and the most frequently used analytical technique, which enables interpretation of the chemical structure of the substance and the determination of its functional groups. Among different IR methods the method of attenuated total reflection (ATR IR), which enables recording of the spectra with high intensity, due to the tight contact between the studied textile and ATR crystal (diamond or germanium), has proven to be the most appropriate method for the study of textiles. When sufficient pressure of textile fibres against an ATR crystal is provided, the fibres flatten and the contact surface between fibres and the ATR crystal increases. The penetration distance of the IR rays, which amounts to a few μm, additionally provides the spectra with high intensity. In this case the IR rays not only decline from the surface of the fibres, but penetrate through the fibres, which additionally strengthen the signal.

Cellulose is a polysaccharide, composed from β-D glucose units. Its properties are most often studied by IR spectroscopy, which helps to determine the degree of crystallinity, the purity of the polymer or the presence of different impurities, as well as structural changes which occur by modification. The process of fibre modification represents an important research field in the textile chemistry. Cellulose fibres can be modified in different ways, chemical pre-treatment, final finishing and biodegradation of cellulose polymer being the important ones.

At the final finishing process the cellulose fibres are coated with a chemical finishing agent, which essentially changes the surface properties of the fibres, providing improved qualites. Multifunctionality and wash fastness of the finish can be achieved by the use of comonomers, which under suitable conditions, form a polymer film on the surface of the fibres. This is enabled by sol-gel technology, which is already used in the most contemporary processes of final finishing in textiles. In this case alkylalkoxysilane (R-Si(OEt)$_3$) are used, where the structure of the group R provides the properties of water resistance, soil resistance, microbial inhibition. Alkylalkoxysilanes, which are brought

manij), snemanje spektrov z visoko intenziteto. Zaradi zadostnega pritiska na tekstilna vlakna ob ATR kristal se vlakna sploščijo in poveča se stična površina med ATR kristalom in preiskovanim substratom. Spektre z visoko intenziteto dodatno zagotavlja tudi prodorna globina IR žarkov, ki znaša nekaj μm. V tem primeru se IR žarki ne odbijejo samo od površine vlaken, temveč prodrejo skozi vlakno, kar še dodatno ojača signal [1].

Celuloza je polisaharid, sestavljen iz β-D glukozidnih enot, katerega lastnosti največkrat proučujemo z IR spektroskopijo. Z njo lahko določimo stopnjo kristalinosti, čistost polimera oziroma prisotnost različnih nečistoč, kot tudi strukturne spremembe pri njeni modifikaciji. V tekstilni kemiji so procesi modifikacije vlaken pomembno raziskovalno področje. Celulozna vlakna lahko modificiramo na različne načine, med katerimi so pomembni postopki kemijske predobdelave, končne apreture ter biorazgradnje celuloznega polimera.

Pri postopkih končne apreture na celulozna vlakna nanesemo kemijska apreturna sredstva, s katerimi bistveno vplivamo na spremembo lastnosti površine vlaken ter jim damo želene funkcionalne lastnosti. Večfunkcionalnost in pralno obstojnost apreture lahko dosežemo z uporabo komonomerov, ki pri ustreznih pogojih na površini vlaken oblikujejo polimerni film. To omogoča tehnologija sol-gel, ki se v tekstilstvu že uporablja pri najsodobnejših postopkih končne apreture. V ta namen uporabimo alkilalkoksisilane (R-Si(OEt)$_3$), pri katerih struktura skupine R pogojuje lastnosti, kot so vodoodbojnost, oljeodbojnost, protimikrobnost. Alkoksisilani, ki se na vlakna nanesejo iz raztopine (sola), se v fazi kondenzacije pri ustreznih pogojih zamrežijo v polisiloksane, ki na površini vlaken tvorijo organsko-anorgansko hibridno nanokompozitno strukturo. Tako oblikovan polimerni film ima debelino le nekaj 10 nm. Nanodelci sola se odlikujejo po odlični adheziji na celulozna vlakna, ki se pri termični obdelavi še dodatno poveča [2].

Pomemben proces, pri katerem pride do strukturnih sprememb v celuloznih vlaknih, je biorazgradnja, ki poteka pod vplivom mikroorganizmov. Medtem ko bakterije razgrajujejo celulozna vlakna s površine proti njihovi notranjosti, pa se njihova razgradnja pod vplivom gliv začne pri razpoki povrhnjice ali na mestih, kjer je vlakno odrezano. Tako lahko spore gliv dosežejo lumen, od koder poteka njihovo nadaljnje razmnoževanje iz notranjosti vlakna v zunanje plasti [3]. Med biorazgradnjo mikroorganizmi sproščajo encime, ki preko reakcij hidrolize in oksidacije povzročijo razpad celuloze do nižjih oligosaharidov, celobioze in glukoze. Delovanje encimov je zelo specifično in strogo definirano. Tako endoglukanaza naključno napade in hidrolizira 1→4 β glukozidno vez, pri čemer nastanejo celo-oligosaharidi. Delovanje endoglukanaze je omejeno predvsem na amorfni del celuloze. Celobiohidrolaza cepi disaharidne ostanke (celobioze) z nereducirajočih koncev celuloznega substrata. V nasprotju z endoglukanazo deluje tako na amorfno kot tudi na kristalino celulozo. Zadnji korak pri razgradnji celuloze je hidroliza celobioze, celotrioze in nižjih oligosaharidov do glu-

to the fabric from the soil, form a polysiloxane network in the condensation process by forming an organic-inorganic hybrid nanocomposite structure on the surface of the fibres. The film formed in this way has a thickness of about 10 nm only. Nanoparticles of the soil are distinguished for excellent adhesion on cellulose fibres, which additionally increases during thermal treatment.

Biodegradation is an important process which leads to structural changes of the cellulose fibres, due to the influence of microorganisms. Although bacteria degrade cellulose fibres from the surface towards the inner parts of the fibres, degradation of fibres by fungi proceeds from the cracks on the surface of the fibres or from the cut-off ends of the fibres. In these ways spores of the fungi reach the lumen, from wich its further multiplication proceeds, that is from the inner towards the outer layer of fibres. In the biodegradation process microorganisms secrete enzymes which cause the decomposition of cellulose to lower oligosaccharides, cellobiose and glucose through the reactions of hydrolysis and oxidation. The mode of action of enzymes is very specific and strictly defined. That is, endoglucanases randomly attack and hydrolyse the 1→ 4 β glucosidic bond to produce cello-oligosaccharides. The activity of endoglucanases is confined to amorphous regions of cellulose only. Celobiohydrolases release disaccharides remains (cellobiose) from the nonreducing ends of a cellulose substrate. Unlike endoglucanases, celobiohydrolases act on amorphous as well as on crystalline cellulose. The last step in the biodegradation of cellulose is hydrolysis of cellobiose, cellotriose and lower oligosaccharides to glucose, which is determined by the action of β-glucosidases. Considering this, the consequences of the biodegradation of cellulose fibres are therefore important changes in the structure of polysaccharide.

koze, kar je pogojeno z delovanjem β-glukozidaze [4]. Glede na to ima biorazgradnja celuloznih vlaken za posledico pomembne spremembe v strukturi polisaharida.

Namen raziskave je bil proučiti uporabo ATR IR spektroskopije za določitev strukturnih sprememb celuloznih vlaken pri njihovi končni apreturi, kot tudi pri biorazgradnji. Pri tem smo predpostavili, da lahko s podrobno spektrofotometrično analizo opišemo lastnosti nanokompozitnega apreturnega filma, in to kljub njegovi majhni debelini, spremembo kemijskih lastnosti oplemenitenih vlaken, kot tudi časovno odvisnost stopnje razgradnje celuloznega polimera pod vplivom mikroorganizmov.

2 Eksperimentalni del

2.1 Podatki o tkanini

V raziskavi smo uporabili tkanino iz 100-odstotnega bombaža v vezavi platno s ploščinsko maso 162 g/m^2 ter gostoto osnove 30 niti/cm in gostoto votka 24 niti/cm. Tkanina je bila pred tem beljena in mercerizirana.

2.2 Aplikacija alkoksisilana na bombažno tkanino

Med alkoksisilani smo uporabili polidimetilsiloksan sečnino (PDMSU) [5] s kemijsko strukturo, prikazano na sliki 1. Pripravili smo 50-odstotno raztopino PDMSU v etanolu, kateri smo dodali 0,1 M HCl, tako da je lahko stekla hidroliza PDMSU. Molarno razmerje PDMSU/H_2O je bilo 1 : 6, in sicer ena molekula vode na vsako skupino OEt. Raztopino smo razredčili do 4-odstotne koncentracije PDMSU in jo nanesli na tkanino po impregnirnem postopku s polnim omakanjem in 15-minutnim toplozračnim sušenjem v laboratorijskem razpenjalnem sušilniku pri temperaturi 140 °C.

2.3 Pralna obstojnost PDMSU apreturnega filma

Pralno obstojnost PDMSU apreturnega filma na bombažni tkanini smo določili s pralnim testom po standardu ISO 105-C01:1989 (E). Pranje smo izvedli v Launder-Ometru v kopelnem razmerju 1 : 50, ob dodatku 5 g/l standardnega pralnega praška, 30 minut pri temperaturi 40 °C ter pH kopeli 7. Po petnajstkratnem pranju smo tkanino večkrat sprali z destilirano vodo ter jo posušili pri sobni temperaturi.

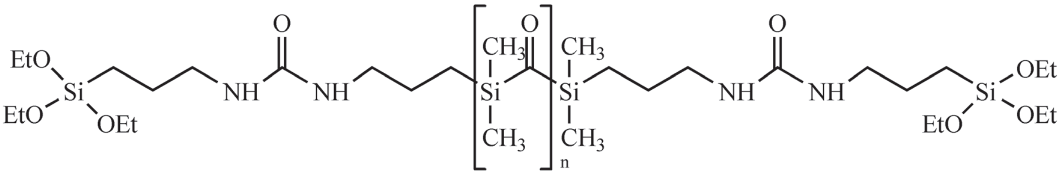

Figure 1: Chemical structure of PDMSU.

The aim of this work was to investigate the use of ATR IR spectroscopy for determining the structural changes of cellulose fibres after final finshing, as well as after biodegradation. At this point, we have presumed that by detailed spectrofotometrical analysis the properties of nanocomposite film, despite its small thickness, the change of chemical properties of modified fibres, as well as the time dependence for the degree of biodegradation of the cellulose polymer by microorganisms can be described.

2 Experimental

2.1 Materials

Plane-wave 100 % cotton fabric with a mass of 162 g/m^2, warp density of 30 yarns/cm and weft density of 24 yarns/cm was used in the experiments. In the pre-treatment processes the fabric was bleached and mercerised.

2.2 Application of alkoxysilane on cotton fabric

Among alkoxysilanes the polydimethilsiloxane urea (PDMSU) [5], with chemical structure shown in figure 1, was used in the experiment. The PDMSU precursor was diluted in ethanole to a concentration of 50 %, whereas its hydrolysis was performed by the addition of 0,5 M HCl. The PDMSU/H_2O molar ratio was 1 : 6, namely, one water molecule for each –OEt group. Less concentrated solution, i.e. 4 %, was used for the impregnation of cotton fabric, which proceeded by full immersion and drying at 150 °C for 15 minutes.

2.3 Wash fastness of PDMSU film

Wash fastness of the PDMSU film on a cotton fabric was determined according to the standard ISO 105-C01:1989 (E) in a Launder-Ometer for 30 minutes. Coated fabric samples were washed for 15 cycles in a soap solution of concentration of 5 g/l at pH 7, previously heated to 40 °C, to give a liquor ratio of 50 : 1. After washing, the sample was rinsed several times in distilled water and dried at room temperature.

2.4 Biorazgradnja bombažne tkanine

Pri biorazgradnji bombažne tkanine smo uporabili test z zakopom po standardu SIST EN ISO 11721-2:2003. Vzorce bombažne tkanine smo za 3, 6, 9 in 12 dni zakopali v humusno ilovico, bogato z mikroorganizmi. Vlažnost zemlje je bila stalna in je znašala 60 ± 5 %, pH je bil med 4,0 in 7,5. Po določenem inkubacijskem času smo proučevane vzorce izkopali iz zemlje, jih rahlo sprali pod tekočo vodo ter jih za 30 minut potopili v 70-odstotni etanol. Po sterilizaciji smo vzorce posušili na zraku, nato pa še sedem ur v sušilniku pri temperaturi 105 °C do konstantne mase.

2.5 FT-IR spektroskopija

FT-IR spektroskopijo proučevanih bombažnih vzorcev smo izvedli na Bruker IFS 66/S spektrofotometeru, opremljenem z ATR celico (SpectraTech). Spektre proučevanih bombažnih vzorcev smo posneli na ATR celici z Ge kristalom (n = 4,0) in na ATR celici z diamantom (n = 2). Spektre smo posneli pri valovnih dolžinah od 4000 do 600 nm.

Pri spektralni analizi vzorcev po določenem času biorazgradnje smo ATR IR spektre le-teh normalizirali na OH trak pri 1200 cm^{-1}, ki je neobčutljiv na spremembe okolice [6].

3 Rezultati z razpravo

Na sliki 2 je prikazan ATR IR spekter proučevane bombažne tkanine. V njem so vidni naslednji trakovi: 3500–3200 cm^{-1}, ki pripada O–H valenčnemu nihanju vode, 2980–2800 cm^{-1}, ki pripada C–H raztezanju, 1640 cm^{-1}, ki je posledica HOH deformacijskega nihanja molekul vode, ter trakovi v spektralnem območju 1500–800 cm^{-1}, ki pripadajo različnim C–H, O–H, C–O in C–O–C nihanjem glukozidnega obroča in so prstni odtis celuloze. Njihova valovna števila so zbrana v preglednici 1 [7–11].

3.1 Spektralna analiza celuloze modificirane s PDMSU

Da bi lahko določili spremembe v kemijski strukturi celuloznih vlaken po njihovem plemenitenju s PDMSU, smo najprej opravili spektralno analizo samega polisiloksanskega filma. ATR IR spekter nehidroliziranega PDMSU, prikazan na sliki 3, jasno razkriva njegovo kemijsko strukturo. Tako v območju 1300–600 cm^{-1} nastanejo trakovi Si–O–Si razteznih nihanj pri 1081 in 1021 cm^{-1} [12, 13], trakovi Si–OEt skupin pri 1166, 1100 in 957 cm^{-1} [14–17] ter trakovi metilnih skupin pri 1260 in 800 cm^{-1} [12,13]. V območju 1800–1300 cm^{-1} nastanejo trakovi, ki pripadajo sečninskim skupinam v PDMSU, značilni tudi za spektre poliamidov in različnih ureasilov [18–21]. S hibridno strukturo ustrezna absorpcijska trakova sta pri valovnem številu 1635 cm^{-1} (Amid I) in pri 1572 cm^{-1} (Amid II).

Na sliki 4 je prikazan ATR IR spekter bombažne tkanine, apretirane s PDMSU. Medtem ko so na njem dobro vidni trakovi PDMSU pri 1260 cm^{-1} (δ(Si–CH_3)), 798 cm^{-1} (r(Si–CH_3)) in CH_3 raztezna niha-

2.4 Biodegradation of cotton fabric

Biodegradation of cotton fabric was carried out by the soil burial test, according to the SIST EN ISO 11721-2:2003 standard. Cotton fabric samples were buried in commercial grade compost, rich in microorganisms, for periods of 3, 6, 9 and 12 days. The water content of the soil was 60 ± 5 % of its maximum moisture retention capacity. It was held constant during the experiment by spraying it with water. The pH of the soil was between 4.0 and 7.5. After the defined incubation time, the samples were removed from the test soil, lightly rinsed with running tap water and immersed in 70 % ethanol for 30 minutes before air drying. Afterwards the samples were dried in an air dryer at 105 °C for 7 hours until constant mass was reached.

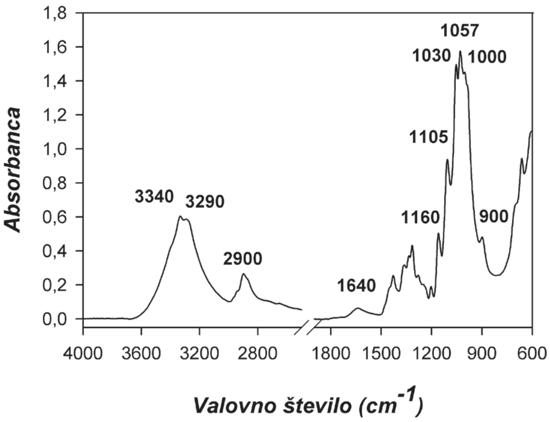

Figure 2: ATR IR spectrum of cotton fabric (for identification of the bands see Table 1).

Table 1: Identification of the bands characteristic for cellulose fibres (ATR IR spectrum).

Wave number [cm^{-1}]	Vibrational mode[a]	Ref.
3550–3100	$\nu(OH\cdots O)$	7–10
2980–2800	$\nu(CH_2)$; $\nu(CH_3)$	
1644	$\delta(OHO)$	7–11
1429	$\delta(CH_2)$; $\delta(CH_3)$	
1368	δ(C–H), $\delta(CH_2)$; $\delta(CH_3)$	
1337*	ν(C–O); δ(OH)	8
1335	$\delta(CH_2)$	7–10
1316	δ(C–H); $\delta(CH_2)$	
1280	$t(CH_2)$	7–9
1235*	δ(C–OH)	
1200*	δ(C–OH); δ(C–CH)	7, 9
1160*	ν_{as}(C–C)	
1105*	ν_{as}(C–O–C)	7–9
1057	Asimetrical stretching of glucosidic ring in plane	8
1030	ν(C–OH) 2° alcohol	7–9
1000–986	ν(C–OH) 1° alcohol	
900	$\nu_{as}(C_1–O–C_4)$	

[a] ν – valence; ν_{as} – valence asymmetrical; ν_s – valence symmetrical; δ – deformational; t – bending.
** the bands belong to connected ν(C–C–C), ν(C–O–C), ν(C–O) and δ(OH) vibrations.*

2.5 FT-IR spectroscopy

The FT-IR spectra of studied cotton samples were obtained on a Brucker IFS 66/S spectrophotometer, equipped with an attenuated total reflection (ATR) cell (SpectraTech). The spectra of studied samples were recorded on an ATR cell with a Ge crystal (n = 4.0) and on an ATR cell with a diamond crystal. The spectra were recorded over the range 4000 – 600 cm^{-1}.

The ATR IR spectra were normalized to the OH absorption band at 1200 cm^{-1}, which is insensitive to environmental changes, in spectral analysis of the studied samples after the defined biodegradation time [6].

3 Results and discussion

In figure 2 the ATR IR spectrum of studied cotton fabric is shown. From the spectrum the following absorption bands could be observed: 3500–3200 cm^{-1}, due to O–H valent vibration of water, 2980–2800 cm^{-1}, due to C–H streaching, 1640 cm^{-1}, due to deformation vibration of water molecules and absorption bands in the 1500–800 cm^{-1} spectral region, which occurred as a result of C–H, O–H, C–O and C–O–C vibration in glucosidic ring and represents the finger print of cellulose. Their wave numbers are shown in table 1 [7–11].

3.1 Spectral analysis of cellulose modified by PDMSU

In order to determine the chemical changes of cellulose after its modification, the spectral analysis of PDMSU film was performed. ATR IR spectrum of nonhydrolyzed PDMSU, shown in figure 3, clearly revealed its chemical structure. Therefore, in the 1300–600 cm^{-1} spectral region absorption bands at 1081 and 1021 cm^{-1} [12, 13], due to Si–O–Si starching vibrations, bands at 1166, 1100 and 957 cm^{-1} [14–17], characteristic for Si–OEt groups and the methyl absorption bands at 1260 and 800 cm^{-1} [12,13] could be seen. In the 1800-1300 cm^{-1} spectral region the bands characteristic for urea moieties in PDMSU were observed. These bands are also found in the spectra of polyamides and various ureasils [18–21]. Absorption bands with corresponding hybrid structure occurred at 1635 cm^{-1} (Amide I) and 1572 cm^{-1} (Amide II).

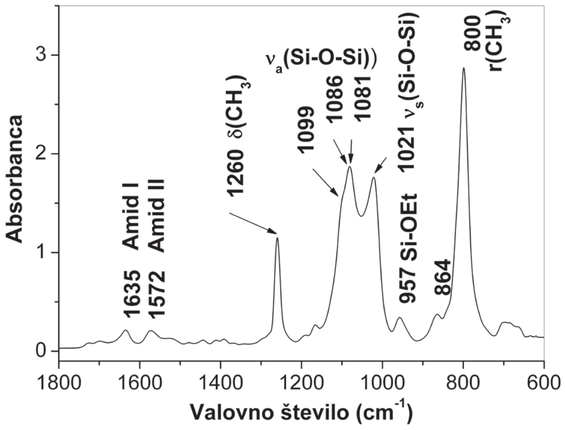

Figure 3: ATR IR spectrum of nonhydrolyzed PDMSU.

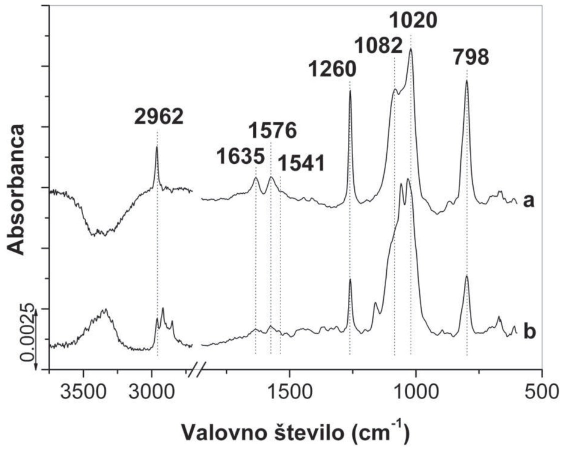

Figure 4: Diferential ATR IR spectrum, given by subtracking the ATR IR spectrum of cotton fabric from the ATR IR spectrum of cotton fabric modified by PDMSU. a) unwashed sample, b) 15 times washed sample.

nja pri 2962 cm^{-1}, pa so raztezna nihanja ν_{as}(Si–O–Si) in ν_s(Si–O–Si) pri 1095 in 1020 cm^{-1}, ki pripadajo poli(dimetilsiloksanski) verigi, zasenčena s trakovi celuloze. Slednja dva trakova (ν_{as}(Si–O–Si) in ν_s(Si–O–Si)) sta lepo izražena na spektru, ki ga dobimo, če spekter neapretirane bombažne tkanine odštejemo od tkanine, apretirane s PDMSU (slika 4). Znatna intenziteta trakov in majhen šum potrjujeta, da lahko kljub majhnemu nanosu PDMSU z analizo ATR IR spektrov na bombažni tkanini določimo kemijske lastnosti proučevane nanokompozitne apreture.

Z analizo ATR IR spektrov lahko spremljamo tudi pralno obstojnost apreturnega filma na celuloznih vlaknih. Iz razlike spektrov, dobljene z odštevanjem spektra neapretirane bombažne tkanine od spektra 15-krat prane apretirane bombažne tkanine, je jasno viden

In figure 4 the ATR IR spectrum of cotton fabric modified by PDMSU is shown. It revealed the bands of PDMSU at 1260 cm^{-1} (δ(Si–CH$_3$)), 798 cm^{-1} (r(Si–CH$_3$)) and the CH$_3$ stretching band at 2962 cm^{-1}, meanwhile the ν_{as}(Si–O–Si) and ν_s(Si–O–Si) stretching bands belonging to the poly(dimethylsiloxane) chain modes at 1095 and 1020 cm^{-1} were blurred by the band of the cotton fabric. The latter two bands were well observed on the spectra obtained by subtracting the spectrum of cotton from the spectrum of cotton modified by PDMSU (figure 4).

By spectral analysis of ATR IR spectra, wash fastness of PDMSU film on cellulose fibres can be studied. From difference spectra obtained by subtracting the spectrum of cotton from the spectra of 15-times washed cotton fabric modified by PDMSU, the course of degradation of the impregnation can be provided. The comparison of spectra a and b in figure 4, namely revealed that repetitive washing caused a decrease of the bands characteristic of PDMSU (2962, 1260, 798 cm^{-1}). The region between 1200 and 1000 cm^{-1} could not be considered in spectral analysis, due to the coincidence of absorption bands of PDMSU and cellulose. In this region the structural changes of PDMSU were obscured by the changes of cotton fabric caused by the ingression and absorption of water as a result of repetitive washing. During washing the hydratation of cellulose as well as PDMSU film occurred, which could be confirmed by the increase of negative intensity band of the ν (H—O···H) stretching mode in the 3500–3000 cm^{-1} region (figure 4b). Furthermore, washing also affected the urea sites in the 1800–1500 cm^{-1} region, as shown in figure 4. By the decrease of intensity of the Amide I band at 1635 cm^{-1} and the Amide II band at 1576 cm^{-1}, the appearance of a new Amide II band at 1576 cm^{-1} could be observed from the spectrum. Meanwhile the decrease of the intensity of the 1635 and 1576 cm^{-1} band reflected the loss of the PDMSU film from the surface of the fibres after repetitive washing, the appearance of the 1541 cm^{-1} band confirmed the breaking or relaxation of the hydrogen bonding between urea groups and most likely between the urea groups and cellulose. Considering this, the washing caused degradation of PDMSU

proces razgradnje PDMSU nanosa po večkratnem pranju. Iz primerjave spektrov a in b na sliki 4 je namreč razvidno, da se po 15-kratnem pranju zmanjša intenziteta trakov, ki so značilni za PDMSU (2962, 1260 in 798 cm^{-1}). Zaradi prekrivanja absorpcijskih trakov PDMSU in celuloze v območju 1200–1000 cm^{-1} le-teh ne moremo vključiti v spektralno analizo. V tem območju so namreč strukturne spremembe PDMSU filma zasenčene s spremembami, ki jih večkratno pranje povzroči na bombažni tkanini in so povezane z adsorpcijo in vstopom molekul vode v celulozna vlakna. Nastanek pozitivnega traku v območju 3500–3000 cm^{-1}, ki pripada ν(H–O···H) razteznim nihanjem vode (slika 4b), kaže na to, da je z večkratnim pranjem prišlo do hidratacije celuloze kot PDMSU filma. Iz slike 4 je tudi razvidno, da je pranje vplivalo tudi na trakove sečnine v območju 1800–1500 cm^{-1}. Ob zmanjšanju intenzitete trakov Amida I pri 1635 cm^{-1} in Amida II pri 1576 cm^{-1} je na spektru jasno viden tudi nov trak Amida II pri 1541 cm^{-1}. Medtem ko zmanjšanje intenzitete trakov pri 1635 in 1576 cm^{-1} kaže na to, da se je PDMSU film pri večkratnem pranju počasi izpiral s površine vlaken, pa pojav traku pri 1541 cm^{-1} potrdi prekinitev ali relaksacijo vodikovih vezi med skupinami sečnine in najverjetneje tudi med sečnino in celulozo. Iz tega lahko sklepamo, da se je med pranjem razgradnja PDMSU filma na bombažni tkanini začela pri molekulah sečnine, kar je vplivalo na njene medmolekulske interakcije s celuloznim substratom.

3.2 Spektralna analiza strukturnih sprememb celuloze pri biorazgradnji

Dosedanje raziskave so pokazale, da pride pri biorazgradnji celuloznih vlaken do znatnih strukturnih sprememb, katerih posledica je znižanje povprečne stopnje polimerizacije in s tem pretržne trdnosti vlaken [22–24]. Te ugotovitve smo potrdili z analizo spektrov, ki je omogočila še dodatno razložiti spremembe v kemijski strukturi celuloze, do katerih je prišlo pod vplivom delovanja mikroorganizmov.

Iz ATR IR spektrov prikazanih na sliki 5, je razvidno, da se z naraščajočim časom biorazgradnje zmanjšuje intenziteta trakov pri 3340 cm^{-1}, ki pripada OH razteznim nihanjem vode, 2918 ter 2853 cm^{-1}, ki jih pripisujemo ν(CH$_2$) in ν(CH$_3$) razteznim nihanjem, kot tudi intenziteta trakov v območju prstnega odtisa celuloze (1500–800 cm^{-1}) pri 1456, 1429, 1370, 1360, 1335, 1315, 1280, 1247, 1235, 1160, 1105, 1057, 1030 in 1000 cm^{-1}, ki izhajajo iz prisotnih C–C, C–H in C–O razteznim nihanjem ter nihanjem glukozidnega obroča [10, 25]. Iz zmanjšanja intenzitete teh trakov, še posebej pa trakov pri valovnih številih 3340, 1429, 1370, 1335 in 1315 cm^{-1}, iz katerih se ocenjuje stopnja kristalinosti [6, 26], smo ugotovili zmanjšanje kristalinosti celuloznih vlaken med biorazgradnjo. Še posebej je značilno tudi zmanjšanje traku pri 900 cm^{-1} (po šestih dnevih) ter njegova razširitev (po 12 dnevih).

Stopnjo kristalinosti ponavadi kvantitativno ovrednotimo z indeksom kristalinosti (I$_k$). V literaturi se najpogosteje omenjajo trije

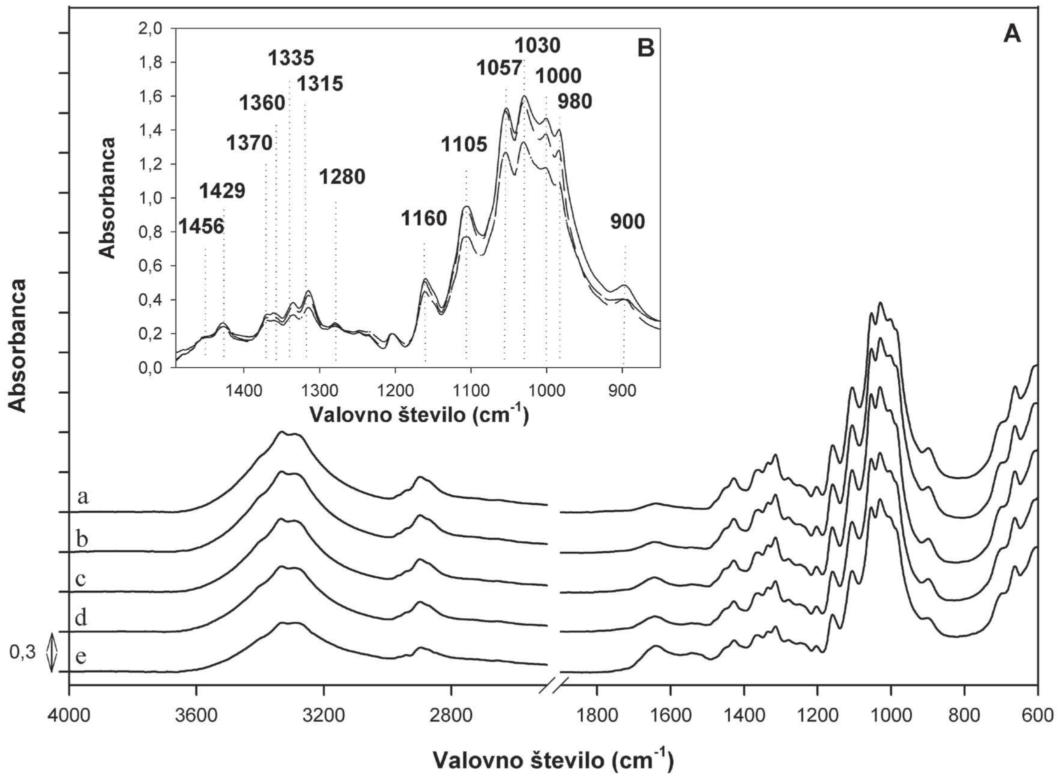

Figure 5: A) ATR IR spectrum of cotton fabric after different biodegradation period. a – 0 day, b – 3 days, c- 6 days, d – 9 days, e – 12 days. B) ATR IR spectrum of cotton fabric after different biodegradation period, normalized to the absorption band at 1200 cm^{-1}: — 0 day, — — 6 days, — · 12 days.

film on cotton fibres, which definitely started at the urea sites and therefore affected the cellulose-urea interface bonding.

3.2 Spectral analysis of structural changes of cellulose after biodegradation

Previous studies have shown that biodegradation of cellulose fibres caused a decrease in degree of polymerization and consequently a decrease in the breaking strength of fibres [22–24]. These findings were confirmed by the spectral analysis, which provided additional explanation of chemical changes of cellulose which occurred by the action of microorganisms.

ATR IR spectra in figure 5 show that by increasing the biodegradation time, the intensity of the bands at 3340 cm^{-1}, due to the OH stretching vibration, 2918 and 2853 cm^{-1}, due to ν(CH$_2$) and ν(CH$_3$) stretching vibration, as

načini določitve vrednosti I_k, in sicer po Nelsonu in O'Connorju [26, 27] iz razmerij jakosti absorpcijskih trakov pri 1429 cm^{-1} (CH$_2$ upogibanje) in 893 cm^{-1} (C–O raztezanje), ter 1372 cm^{-1} (nihanje C–H skupine celuloznega obroča) in 2900 cm^{-1} (C-H raztezanje), ter po Hullemanu [6] iz razmerja jakosti absorpcijskih trakov pri 1280 cm^{-1} (CH upogibanje) in 1200 cm^{-1} (–OH upogibanje). Čeprav se ti načini uporabljajo predvsem za določitev spremembe stopnje kristalinosti celuloze pri njenih transformacijah iz ene v drugo kristalno modifikacijo, smo jih s pridom uporabili tudi za spremljanje biorazgranje celuloze. Presenetljivo (slika 6) vrednost I_k, ne glede na način njene določitve [6, 26, 27] narašča z naraščajočim časom zakopa. To pomeni povečevanje stopnje kristalinosti, in to kljub temu, da se kristalinost celuloze med biorazgradnjo zmanjša. Ti rezultati so smiselni, saj mikroorganizmi hkrati napadejo in razgrajujejo tako amorfni kot kristalini del celuloze. Hitrejša razgradnja manj urejenega amorfnega dela celuloze, ki omogoča lažje in hitrejše prodiranje encimov v primerjavi z visoko urejenim kristalinim delom celuloze, pa je lahko vzrok za povečevanje vrednosti I_k z naraščajočim časom biorazgradnje [4].

Encimi, ki jih sproščajo bakterije in glive med biorazgradnjo, *well the intensity of the bands in the cellulose finger print region (1500–800cm*$^{-1}$*) at 1456, 1429, 1370, 1360, 1335, 1315, 1280, 1247, 1235, 1160, 1105, 1057, 1030 and 1000 cm*$^{-1}$*, due to the C–C, C–H and C–O stretching vibration and vibration of glucosidic ring, decreased [10, 25]. The decrease in these bands, especially the bands at 3340, 1429, 1370, 1335 and 1315 cm*$^{-1}$*, from which the degree of crystallinity can be evaluated [6, 26], indicated that the degree of crystallinity of cellulose fibres decreased during biodegradation. This additionally confirmed the characteristic decrease in the 900 cm*$^{-1}$ *band (after 6 days) and its broadening (after 12 days).*

The degree of crystallinity is usually quantitatively evaluated by the crystallinity index (I_k*). In literature three methods of* I_k *determination are most often documented, namely by the Nelson and O'Connor method [26, 27] from the proportion of the intensity of absorption band at 1429 cm*$^{-1}$ *(*CH_2 *bending) and 893 cm*$^{-1}$ *(C–O stretching) as well as from the 1372 cm*$^{-1}$ *(vibration of C–H group of cellulose ring) and 2900 cm*$^{-1}$ *(C–H stretching), and by the Hulleman method [6] from the proportion of the intensity of the absorption bands at 1280 cm*$^{-1}$ *(CH bending) and 1200 cm*$^{-1}$ *(–OH bending). For the study of biodegradation of cellulose these methods were used despite the fact that they are mostly used for the determination of changes of degree of crystallinity of cellulose after its transformation from one into another crystalline modification. Surprisingly, the values of* I_k *increased with increasing the biodegradation time, in spite of the method used for its determination. This meant that the degree of crystallinity increased despite the fact that crystallinity of cellulose decreased after the biodegradation process. These results can be explained since microorganisms simultaneously attack and degrade the amorphous as well as crystalline region of cellulose. A quicker degradation of less ordered amorphous region of cellulose, which enables easier and faster penetration of microorganisms in comparison to the well ordered crystalline region of cellulose, could be the reason for increasing the* I_k *values with increasing the biodegradation time [4].*

During the biodegradation enzymes produced

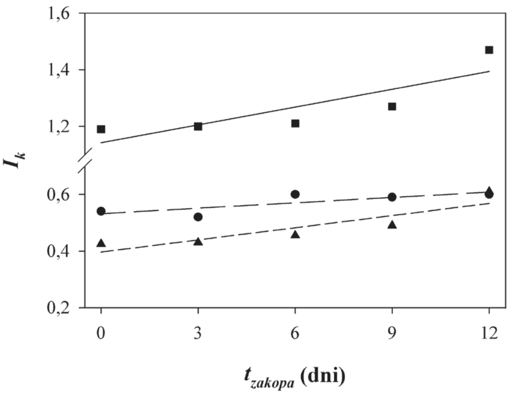

Figure 6: Dependence of crystallinity index, I_k*, from burial time of cotton fabric sample. Method of* I_k *determination: -●- proportion of intensity of the bands at 1429 and 898 cm*$^{-1}$*, -■- proportion of intensity of the bands at 1372 and 2900 cm*$^{-1}$*, -►- proportion of intensity of the bands at 1280 and 1200 cm*$^{-1}$*.*

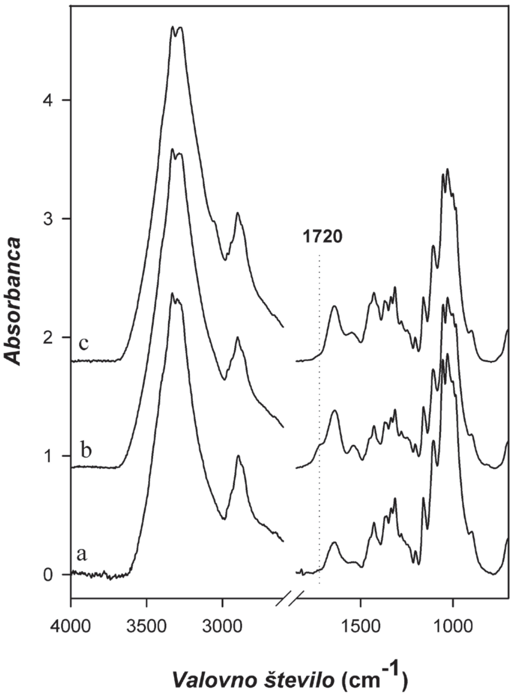

Figure 7: ATR IR spectrum of buried cotton fabric. a – untreated sample, b – sample treated with HCl vapor, c – sample treated with NH_3 *vapor.*

povzročijo tako hidrolitično kot oksidativno razgradnjo celuloze. Medtem ko ima hidrolitična razgradnja celuloze za posledico cepitev 1→4 β glukozidne vezi ter tvorbo aldehidnih skupin, se pri *by various bacteria and fungi in soil catalyse the hydrolytic as well as oxidative degradation of cellulose macromolecules. While the hydrolytic degradation results in the breaking of (1→4) β-glycosidic bonds, and the occurrence of the formation of aldehyde groups; the oxidative degradation of cellulose results in the opening of the β-D-glucopyranose rings, causing the formation of carboxylic as well as aldehyde groups [4]. These bands are located in the region from 1750 to 1617 cm*$^{-1}$ *[11, 28]. The presence of carboxylic groups in the structure of degraded cellulose was proved by a few minutes' exposure of fabric to HCl vapour. In an HCl atmosphere, the protonation of carboxylate groups results in the rise of the absorption band at 1720 cm*$^{-1}$*, which disappears again when the fabric is exposed to the* NH_3 *vapour (Figure 7). The presence of hydrolytic and oxidative damages of cellulose was also confirmed by the decrease of intensity of bands in the 1150–900 cm*$^{-1}$ *region, due to a decreased number of 1→4 β glucosidic bond and β-D-glucopiranose ring, as well as a decrease in the band at 2900 cm*$^{-1}$*, resulting from a decreased number of CH groups.*

A comparison of the ATR IR spectra of cellulose after different biodegradation periods (figure 8) also showed that the intensity of the band at 1640 cm^{-1} *increased with the increased burial time. Simultaneously a new band at 1548 cm*$^{-1}$ *appeared beside it. The position and shape of these bands were not representative for the spectral absorption of the aldehyde or carboxylic functional groups which are produced during the microbial degradation of cellulose. They seem to be more related to the Amides I and II [29]. Their appearance in the cellulose structure could be explained by the presence of secondary polyamides originating from the proteins which are produced during the growth of microorganisms on the fibres, and could be irreversibly adsorbed. Therefore, greater microbial growth on cotton fibres resulted in an increase of the absorption bands at 1640 and 1548 cm*$^{-1}$ *and increased with the time of burial, which consequently reflected in a greater biodegradation.*

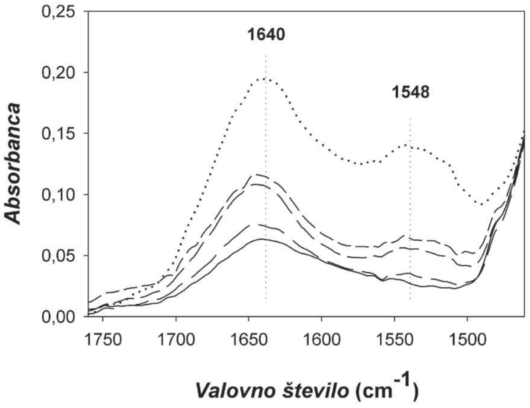

Figure 8: ATR IR spectrum of cotton fabric after different biodegradation period in the 1760 – 1460 cm^{-1} *region. — 0 day, — — 3 days – – 6 days, --- 9 days, ···· 12 days*

oksidativni razgradnji cepi β-D-glukopiranozni obroč, pri čemer nastanejo tako aldehidne kot karboksilne skupine [4]. Le-te imajo trak v območju 1750–1617 cm^{-1} [11, 28]. Prisotnost karboksilnih skupin v strukturi razgrajene celuloze smo dokazali z nekajminutno obdelavo bombažne tkanine s HCl hlapi [8]. Pri tem je prišlo do protonacije karboksilne skupine, zaradi česar je nastal nov trak pri valovnem številu 1720 cm^{-1}. Slednji je ponovno izginil, ko smo tkanino izpostavili hlapom NH_3 (slika 7). Prisotnost hidrolitičnih in oksidacijskih poškodb celuloze potrjuje tudi zmanjšanje intenzitete trakov v območju 1150–900 cm^{-1} zaradi zmanjšanja števila 1→4 β glukozidnih vezi ter β-D-glukopiranoznih obročev in zmanjšanje traku pri 2900 cm^{-1}, ki je posledica zmanjšanja števila CH skupin (slika 4).

Iz primerjave ATR IR spektrov celuloze po različnih časih biodegradacije (slika 8) je tudi razvidno, da intenziteta traku pri valovni dolžini 1640 cm^{-1} narašča s časom zakopa, ob njem pa nastane tudi nov trak pri 1548 cm^{-1}. Tako lega kot tudi oblika obeh trakov ni tipična za aldehidno oziroma karboksilno skupino, ki nastaneta med mikrobno razgradnjo. Veliko bolj ustrezata trakovom Amida I in Amida II [29]. Njun pojav v spektru celuloze smo razložili s prisotnostjo sekundarnih poliamidov, izvirajočih iz proteinov, ki nastajajo med rastjo mikroorganizmov in so se ireverzibilno adsorbirali na celulozna vlakna [28] . Večanje stopnje rasti mikroorganizmov na bombažnih vlaknih se odraža z večanjem intenzitete absorpcijskih trakov pri 1640 in 1548 cm^{-1} in se povečuje s časom zakopa, kar kaže na povečanje biorazgradnje.

4 Conclusions

In the research, the possibility of the use of ATR IR spectroscopy in the study of structural changes of cellulose fibres was studied. On the basis of spectral analysis of cotton fabric modified by PDMSU the bands characteristic for nanocomposite impregnation were determined. From the changes in intensity of these bands after repetitive washing the degree of nanocomposite film degradation was evaluated. The changes in cellulose macromolecule, which occurred during biodegradation process, were studied by ATR IR spectroscopy. On the basis of intensity changes of the bands in the cellulose finger print regions the crystallinity change of the fibres was determined. The presence of carboxylic groups resulting from hydrolytic damage of the fibres was also proven. The appearance of new bands, characteristic for Amide I and Amide II, were explained by the microbial growth on degraded fibres.

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4 Sklepi

V raziskavi smo proučili možnost uporabe ATR IR spektroskopije pri študiju strukturnih sprememb celuloznih vlaken. Na podlagi spektralne analize bombažne tkanine, apretirane s PDMSU, smo določili trakove, značilne za nanokompozitno prevleko. Iz spremembe intenzitete teh trakov po večkratnem pranju smo sklepali na stopnjo razgradnje nanokompozitnega filma. Z ATR IR spektroskopijo smo natančno proučili spremembe v makromolekuli celuloze, ki so nastale med biorazgradnjo. Na podlagi spremembe intenzitete trakov v območju prstnega odtisa celuloze po različnih časih biorazgradnje smo določili spremembo kristalinosti vlaken. Prav tako smo dokazali prisotnost karboksilnih skupin kot posledico hidrolitičnih poškodb vlaken. Na novo nastala trakova, značilna za Amid I in Amid II, smo razložili z rastjo mikroorganizmov na razgrajenih vlaknih.

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