Simona Strnad[1)], Maja Paš[2)], Alenka Fabjančič[2)], Peter Raspor[2)]

Antifungal Activity Assessment of Cotton Fabrics Using Image Processing and Analysis

[1)] **University of Maribor, Faculty of Mechanical Engineering, Institute for Engineering Materials and Design, Characterization and Processing of Polymers Laboratory,**
Slovenia, Simona.strnad@uni-mb.si,
Phone: +38622207882, Fax: +38622207990

[2)] **University of Ljubljana, Biotechnical Faculty, Food Science and Technology Department, Chair of Biotechnology,**
Jamnikarjeva 101, 1000 Ljubljana, Slovenia,
maja.pas@bf.uni-lj.si,
peter.raspor@bf.uni-lj.si

Abstract
An investigation into the antifungal activities of anti-microbially, hydrophobically and oleophobically finished cotton fabrics designed for tents and marquees was performed according to the AATCC 30-1999 (IV) test method. The growth of test moulds (Aspergillus niger, Penicilium pinophilum and Trichoderma harzianum) on differently treated cotton fabric samples was evaluated according to the standard procedure and additionally using image processing and analysis, which have, to a great extent, increased the accuracy of standard organoleptic estimation. Image processing and analysis enabled an exact evaluation of tests of microorganism growth over a percentage area of a fabric sample covered by fungi, after a defined exposure period. The introduction of an evaluation technique using image processing and analysis was shown to be suitable for the purpose of assessing fungal attack. The results of the tests performed on selected antifungal compounds showed significant distinctions even between those cotton fabric samples treated with different amounts of antifungal agents.

Key words: *cotton, antifungal activity, antimicrobial tests, image processing and analysis.*

Inroduction

All surfaces in natural and artificial environments are covered by ubiquitous microorganisms [1]. Microorganisms can cause problems in textile raw materials, process chemicals, during wet textile treatments, in textile and textile product warehouses during transportation, and even during the everyday usage of textile products [2, 3]. Fungi produce at least three enzymes ('cellulase complex') of extra cellular activity [4, 5] during growth on exoglucanase or ß-D-glucosydases cellulose, respectively, remove disaccharide units from the chain ends of endoglucanases or ß-D-glucanohydrolases cellulose, respectively, and randomly break the ß-1,4 bonds of cellulose chains; the third component is ß-D-glucosidases, hydrolysing cellobiose into glucose units, which are then used as a carbon source for fungal growth [6-8]. On cotton fabric, fungal contamination starts on the cutting edge where the spores can easily reach the fibre's lumen [6]. Hyphaes sprout in the lumen and form a mycelium, which grows toward the fibre's wall, causing its degradation.

The damage caused by microorganisms becomes visible with changes on the textile or fibre surface, mostly in the form of de-coloration and stains; in most cases these changes are followed by a typically musty smell. De-coloration is mainly caused by chemical reactions between metabolites secreted by the microorganism and finishing agents or dyes in the textile material. In many cases this leads to the production of pigment-like substances [4, 6].

There are a lot of standardised methods for the antifungal activity assessment of textiles, which mainly prescribe a visual estimation of fungal growth on textile samples or their de-coloration [9]. In some cases, where mechanical properties are the most important, test methods also include the determining of the mechanical properties of textile samples before and after an attack of microorganisms. Certain quantitative approaches, e.g. according to Quin [10], include the application of a high performance microscope for counting bacteria or fungal colonies; such manual microscope measurements are very slow and tedious; however, image processing and analysis solves these problems very successfully. Image processing is a tool that converts analogue information of the image into a numerical form and is usually used for two somewhat different purposes [11]: firstly, for improving the visual appearance of images for the viewer, and secondly for preparing images to measure features and structures. Such image processing and analysis has been increasingly introduced into fields where "organoleptic" evaluation or so-called "manual" data acquisition are the only ways to acquire analysis results. In textile research, image processing and analysis have been mainly used to measure cotton fibre maturity [12, 13] or to determine the distribution of the diameter of wool fiber [14]. There have also been some approaches using flat-textile porosity measurements. However, the application of image analysis as a tool for quality control and textile damage determination has increased over the past few decades [15, 16]. Computer processing is a method of obtaining numerical information from images which is more accurate, less time-consuming and more reproducible than other methods [17].

The aim of the present research was to determine the antifungal activities of special cotton fabric samples for tents and marquees that had been treated with antifungal agents and flame-retardant, oleo- and hydro-phobic agents in the same finishing bath. The influence of interactions between different agents on the antifungal activity of a treated fabric was investigated. Several methods for the antifungal activity assessment of the textile samples were studied, and Test IV of the AATCC 30-1999 method was chosen [18] as the most appropriate for these types of specialty textiles. The results of the conventional visual technique for fun-

gal growth evaluation anticipated by the standard test method were compared to the image analysis results. Image processing and analysis even enabled to distinguish between cotton samples treated with different amounts of the antifungal agent.

Experimental

Materials

Fabric samples

Cotton fabric of high thread density (warp: 335 threads/10 cm and weft: 195 threads/10 cm) and high weight per unit area (280 g/m^2), designed for tents and marquees, produced by Induplati Ltd., was used during this research.

A standard finishing solution composition was proposed by the producer in order to obtain antimicrobial properties as well as oil- and water- repellency. An impregnation procedure was applied using an antimicrobial triazol compound (Rucobac EPV) [19, 20], oil- and water-repellency fluorocarbon polymer (Rucoguard AFB), and a polyisocyanate fixing agent (Rucoguard NET). In order to investigate the influences of the agents applied on the fabric's antimicrobial properties, several samples were prepared using different recipes for preparing the impregnation solution (***Table 1***).

Experimental impregnation procedures were set up according to statistical rules. The last four samples were repetitions of the impregnation procedure, in which the same solution composition was used in order to evaluate standard deviations. Four parallel test samples: A, B, C and D were taken for the assessment of antimicrobial properties. The control sample (K) was an untreated raw fabric sample.

Test organisms

The following test organisms were used in the antifungal activity assay: *Trichoderma harzianum* (CBS 370.52), *Penicillium pinophilum* (CBS 631.66) (List of cultures, 1990; http://www.cbs.knaw.nl/databases/index.htm) and *Aspergillus niger* (Collection of industrial microorganisms, F42). During the experiment, the cultures were kept on agar plates and slants in an incubator at a temperature of 301K.

Culture medium

Malt agar was applied for the safekeeping and growth of the *Trichoderma harzianum* bioculture, and potato dextrose agar – for the *P. pinophilum* and *A. niger* cultures.

Table 1. *Finishing solution composition and sample denotation.*

Test sample	Finishing procedure	Finishing solution composition		
		Antimicrobial agent, g/l	Oil- and water repellency agent, g/l	Auxiliary agent, g/l
A, B, C, D	K (control)	–	–	–
A, B, C, D	1	80	50	50
A, B, C, D	2	30	50	50
A, B, C, D	3	80	5	50
A, B, C, D	4	30	5	50
A, B, C, D	5	80	50	10
A, B, C, D	6	30	50	10
A, B, C, D	7	80	5	10
A, B, C, D	8	30	5	10
A, B, C, D	9	49	15,8	22,4
A, B, C, D	10	49	15,8	22,4
A, B, C, D	11	49	15,8	22,4
A, B, C, D	12	49	15,8	22,4

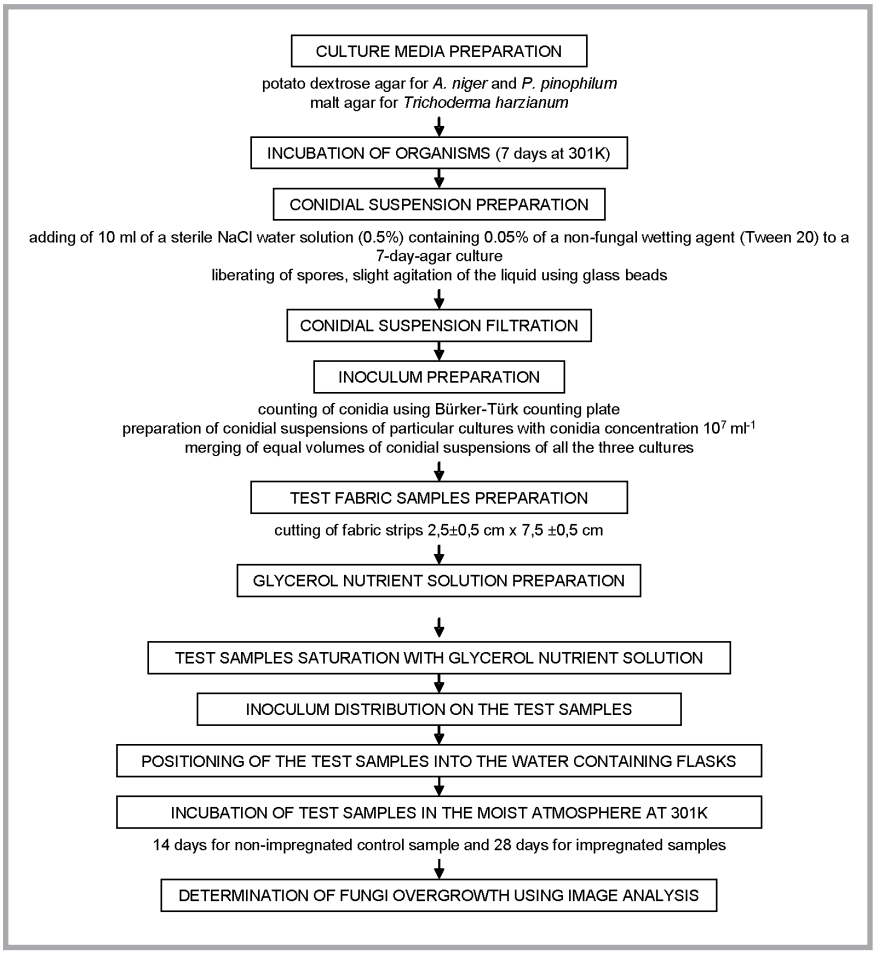

Figure 1. *Schematic presentation of antifungal activity assessment.*

Methods

The antifungal activity assessment of the finished cotton fabric samples was performed according to test IV of the AATCC Test Method 30-1999. A schematic presentation of the experimental work is presented in ***Figure 1***.

The samples were evaluated every 7 days over a 28 day incubation period. Visual and microscopic estimation of the samples' appearance was performed according to the AATCC standard test method using three estimation levels: 0 – no growth, 1 – microscopic growth

```
1.  imgdelete "***"
2.  Gclear 0
3.  imgload "c:\ks300\conf\images\fungi_sample.jpg",1
4.  wincopy 1,2,64,592,1916,606,64,592
5.  imgload "c:\ks300\conf\images\fungi_nontreated_sample.jpg",3
6.  wincopy 3,4,64,522,1916,606,64,592
7.  divide 2,4,5,170
8.  scalint 5,6,129,217,0,255
9.  median 6,7,7
10. dislevrgb 7,8,1,0,59,156,8,98,0,99,10,"RGB"
11. binscrap 8,9,0,40,0
12. binerode 9,10,7,1
13. bindilate 10,11,7,1
14. binfill 11,12
15. MSsetfeat "FIELDFEAT"
16. MSsetframe
17. MSmeasmask 12,1,"DATABASE",0,1,10
18. MSsetfeat "DRAWFEAT"
19. MSdrawmask 12,1
20. Gmerge 1,255
```

Figure 2. *Image of the processing and analysis protocol.*

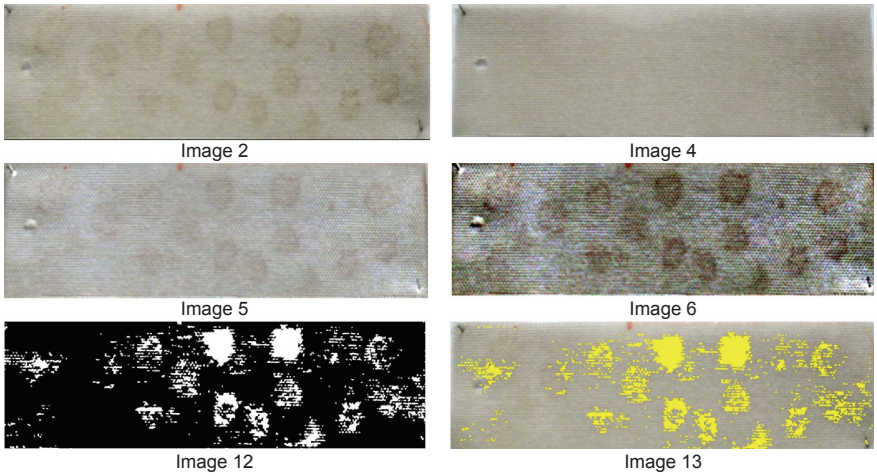

Figure 3. *Resulting images of single processing steps.*

Table 2. *Fungi growth on fabric samples estimated after 28 days of incubation according to AATCC 30-1999 (IV): 0 – no growth, 1 – microscopic growth (visible only under a microscope at 50x magnification), 2 – macroscopic growth (visible to the naked eye).*

Sample	A	B	C	D	average
K (control)	2	2	2	2	2
1	1	2	2	2	2
2	2	2	2	2	2
3	2	1	1	2	1-2
4	2	2	2	2	2
5	2	1	1	1	1
6	2	2	1	2	2
7	2	2	1	1	1-2
8	2	2	2	2	2
9	2	1	2	1	1-2
10	2	1	2	2	2
11	2	2	2	2	2
12	2	2	2	2	2

(visible only under a microscope at 50x magnification), and 2 – macroscopic growth (visible to the naked eye). Microscopy was also used to confirm the assumption that all darker spots on the treated fabric samples were due to fungal activity.

After 28 days of incubation, the samples were photographed under defined exposure conditions using a high-resolution digital photo camera. Photographs of both sides of the samples were taken (a – side of inoculum distribution and b – the other side) in order to follow the growth of fungi throughout the time periods of the fabric samples.

Image analysis

Images of the fabric surfaces were analysed using KS 300 Rel. 3.0 software in the "true colour" mode (Zeiss) for image processing and analysis, and the area % of fungal overgrowth determined.

Special protocol was designed to ensure the exact same measuring procedure for all the specimens, (***Figure 2***). Subjectivity in the evaluation was excluded by respecting this protocol.

The images were loaded by the *"imgload"* command and by the *"wincopy"* command, and the exact dimensions of the images evaluated were defined. Two images were loaded for each analysis: an image of the sample analysed , which had been exposed to fungi growth (image 2 in ***Figure 3***), and an image of the so-called blank (non-exposed) sample (image 4 in ***Figure 3***). Normalisation of the background was performed by the division of image 4 from image 2, the result being image 5 in ***Figure 3***. The *"scalint"* command was applied for image contrast improvement (image 6 in ***Figure 3***) and *"dislevrgb"* for colour segmentation. The segmentation command produced a so-called binary image, which was additionally processed by commands 11, 12, 13 and 14, in order to achieve the best possible definition of the features measured – the area occupied by the fungi. The result of the binary image processing was image 12 in ***Figure 3***, and image 13 in ***Figure 3*** represents the processing procedure's efficiency, with the bright regions in the image representing the area occupied by the fungi.

Results and discussion

Table 2 presents results for the standard visual estimation of fungal growth on the fabric samples, according to the AATCC 30-1999 (IV) method. For the majority of samples, the effects of the fungal presence and growth are visible to the naked eye, hence estimation value 2 prevails. For the samples treated with larger amounts of the antimicrobial agent (samples 3, 5 & 7), the effects of fungal growth are visible only under a microscope, and it is

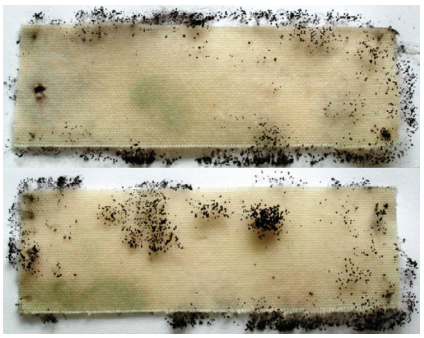

Figure 4. *Control sample (K) after 28 days of incubation.*

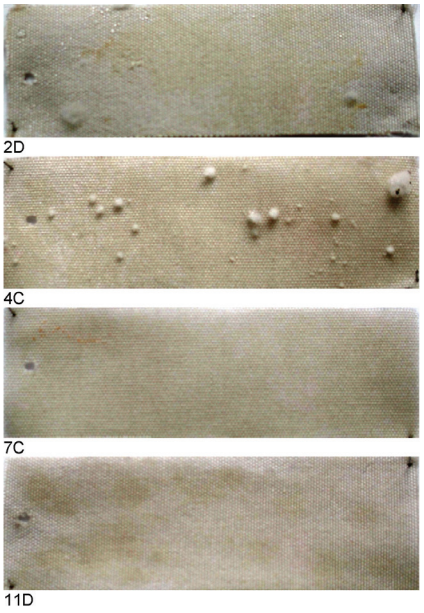

Figure 5. *Selected examples of the appearance of the fabric samples after 28 days of incubation.*

very difficult or practically impossible to make a clear distinction between some of them using a visual (analogous) evaluation technique.

The non-treated control sample (K) behaved totally differently in comparison to the other anti-microbially finished samples during the performance of the tests of antifungal activity. All three test moulds applied were visible on that particular sample. The yellowish-green stains of *Trichoderma harzianum* are visible on the control sample (***Figure 4***) but are absent on all the other finished fabric samples (***Figure 5***). On the control sample the mycelium and conidia of *Aspergillus niger* are also clearly visible; but they are mainly limited to the edges of the sample owing to the cut fibre edges, which are more sensitive to fungal attack. *Penicillium pinophilum* is also present in a higher amount on the control sample but is mainly covered by *Aspergillus niger* (***Figure 4***).

The mycelium with the conidia of *Aspergillus niger* is absent on the fabric samples antimicrobially and oil- and water repellent finished (***Figure 5***). However, under a microscope it was established that the darker stains on these samples were caused by those particular fungi. The changed behaviour – growth and stains, could be caused by the chemical reaction of the metabolite secreted by the fungi, or by the chemicals with which the fabric was treated. Owing to the absence of a conventional form of *Aspergillus niger*, the colonies of *Penicillium pinophilum* are also more easily visible on the finished fabric samples (samples 2 & 4 in ***Figure 5***).

The inoculum distribution on those samples treated with a water-repellent agent (a hydrophobic surface) was difficult to determine owing to the build-up of inoculum drops on the fabric' surfaces, the reason for which being that the inoculum was mechanically smeared over the surface, for which a special rounded-hook was used. Despite the careful procedure for inoculum distribution on the surfaces of those samples treated with high concentrations of the water-repellent agent (samples 1, 2, 5 & 6), drops built-up, and therefore fungal growth also occurred in certain forms on the surface.

The results regarding the area % of fungal overgrowth, determined using image processing and analysis, are represented on the diagram in ***Figure 6***. There are significant differences in the area % of fungal overgrowth between the control sample, where the area amounts to approx. 40%, and all the other chemically-treated samples, where the area covered by fungi is smaller than 18%. Hence, it follows that all the treatment procedures applied inhibit fungal growth significantly. It can also be concluded from the diagram in ***Figure 5*** that the estimation method using image processing and analysis is, in comparison to the standard visual estimation procedure, also sensitive enough to distinguish between those fabric samples treated with different amounts of the antimicrobial agent.

Samples 2, 4 and 8 exhibit slightly weaker antimicrobial activity in comparison to the other chemically treated samples. The area % covered by fungi on those samples amounts to between 15.8% and 18.2%, thus all of them were treated with lower amounts of the antifungal agent. However, sample 6, which was also treated with the same amount of the antifungal agent, did not demonstrate such low antifungal activity. Presumably, in this case a higher amount of the oil- and water-repellent agent caused poor wetting with inoculum and, as such, a poor conidial suspension spread.

Samples 1, 3, 5 and 7 were treated with high amounts of the antifungal agent (80 g/l) and, as such, exhibited higher antifungal activity. The area % of fungal overgrowth on this group of samples is approx. 10% or lower, and no significant differences could be noticed between them. Hence it can be concluded that the oil- and water-repellent agents had a lower influence on fungal overgrowth, as was in the case of lower concentrations

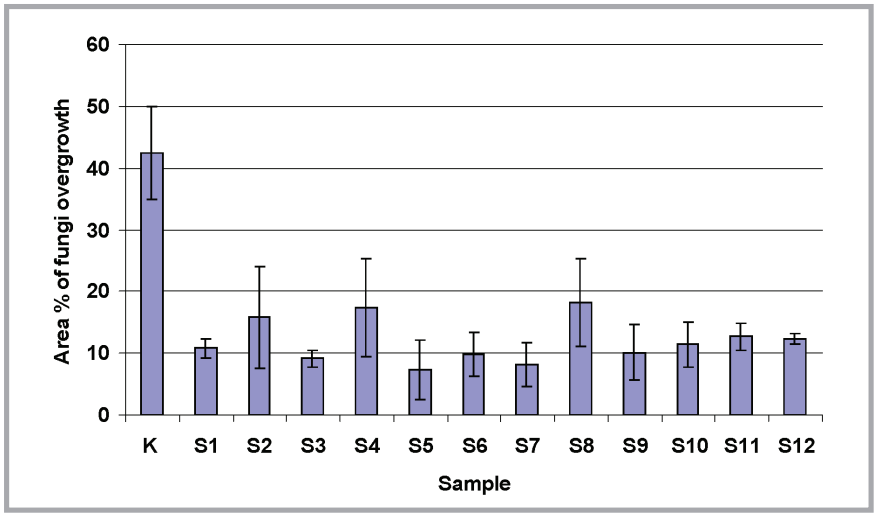

Figure 6. *Area % of fungal overgrowth of the control (K) and differently treated samples (S1 to S12).*

of the antimicrobial agent. This conclusion can be proved by comparing sample pairs 6 and 8 with 5 and 7. Between samples 5 and 7 there is no significant difference in the area % of fungal overgrowth, amounting to 7.3% and 8.1%, despite different concentrations of the oil- and water-repellent agent in the finishing bath (50 & 5 g/l). However, in the case of samples 6 and 8, treated with the lowest concentration of the antimicrobial agent (30 g/l), the area % of fungal overgrowth is significantly different (9.7% & 18.0%).

In order to test for systematic errors, samples 9, 10, 11 and 12 were treated in finishing baths with the same chemical composition. On the basis of a significant analysis (t-test), it was established that there were significant differences between the untreated (control) sample and all the treated sample groups ($p < 0,0002$). The evaluation technique introduced, in which image processing and analysis were used, also proved sensitive enough to trace significant differences in the inhibitory effects of the antimicrobially treated samples with a higher concentration of the antimicrobial agent (80 g/l), in comparison to those treated with a lower concentration (30 g/l) ($p = 0,020$).

The influence of added amounts of oil- and water-repellent agents had a significant influence on the antimicrobial effect of the treated fabric samples only in the case of lower concentrations of the antimicrobial agent in the finishing bath. In this case the hydrophobic agent increased the inhibitory effect of the antimicrobial agent. However, the presence of an auxiliary agent had, in all cases, an inhibitory effect on the antimicrobial properties of the fabric samples.

Summary

The aim of the research presented was to determine the antifungal activity of special cotton fabric samples for tents and marquees treated with antifungal as well as oleophobic and hydrophobic agents, using the same finishing procedure. Test IV of the AATCC 30-1999 method was chosen as the most appropriate for testing these types of samples. Three test organisms were applied according to the test method: *Aspergillus niger*, *Penicilium pinophilum* and *Trichoderma harzianum*.

Image processing and analysis were successfully introduced for the evaluation of fungal growth in order to distinguish small differences between different antimicrobially finished samples, the results of which were compared to those using the standard visual estimation technique.

All the test fungi applied could be traced in large amounts on the non- antimicrobially treated control sample after 28 days of incubation, which did not occur in the case of the antimicrobially treated samples. On the antimicrobially treated samples, mycelium with the conidia of *Aspergillus niger* was absent; there were only darker stains, which were the products of the fungi's changed behaviour, presumably owing to the chemical reactions of the metabolite secreted by the fungi, as well as to the finishing agents present on the surface.

The standard visual (organoleptic) evaluation method for fungal growth on fabric samples allowed to clearly distinguish between the appearance of the non-treated control sample and all the other anti-microbially treated samples. Differences between the various anti-microbially treated samples, however, were indistinguishable using the standard estimation method.

The image processing and analysis method, which was successfully introduced during this research, proved sensitive enough to differentiate between samples treated with different amounts of the antimicrobial agent. The results show that those treatments using large amounts of the antimicrobial agent (80 g/l) caused a significantly higher inhibitory effect on fungal overgrowth (area % covered by fungi amounted to 18% and higher) than those samples treated with smaller amounts (30 g/l) of that particular agent (the area % covered by fungi amounted to 10.8 % or lower). The presence of oil- and water-repellent agents had a significant influence on the antifungal activity of the samples tested only in the cases of smaller amounts of the antimicrobial agent in the finishing bath. The presence of an auxiliary agent displayed a negative influence on the anti-fungal properties of the samples tested.

The standard evaluation method, which is prescribed in the AATCC 30-1999 method (Test IV), is easily applicable for determining differences in the fungistatic effectiveness of anti-fungally treated and non-treated fabric samples. However, when determining the fungistatic effectiveness of different anti-fungally-treated fabric samples, the visual evaluation method was shown to be lacking in sensitivity. The evaluation technique introduced, in which image processing and analysis was used, proved extremely promising.

References

1. Gu J.D., International Biodeterioration & Biodegradation, 52, 1 (2003).
2. Žnidarec T., Jarc O., Tekstilec, 46, 5-6 (2003).
3. Hamlyn P.F., McCarthy B.J., Biologist, 47, 4 (2000).
4. Montegut D., Indictor N., Koestler R. J., International Biodeterioration, 28, 1-4 (1991).
5. Nevalainem H., Pentillä M., "The Mycota" (K. Esser Ed., U. Kück vol. Ed.), vol. 2, pp. 303-319, Berlin, Springer Verlag, 1995.
6. Zyska B., International Biodeterioration & Biodegradation, 53, 3 (2004).
7. Mandels M., Reese E.T., Journal of Industrial Microbiology & Biotechnology, 22, 6 (1999).
8. Walker L.P., Wilson D.B., Bioresource Technology, 36, 1, (1991).
9. Vigo T.L., "Textile processing and Properties: Preparation, Dyeing, Finishing and Performance", 2nd imp. Amsterdam, Elsevier, 1997.
10. Vigo T. L. in "Handbook of Fibre Science and Technology" (M. Lewin, S. Selo Eds.) vol. 2, Part A, pp. 367-426, Marcel Dekker Inc. New York, 1983.
11. Russ J. C., "The Image Processing Handbook", 2nd Ed., pp.1-49, CRC Press, Boca Raton, Ann Arbor, London-Tokyo, 1995.
12. Boylston E. K., Thibodeaux D. P., Evans J. P., Textile Research Journal, 63, 2 (1993).
13. Wu Y., Pourdeyhimi B., Spivak S. M., Textile Research Journal, 61, 7, (1991).
14. Baxter B. P., Brims M. A., Taylor T. B., J. Text. Inst. 83, 4 (1992).
15. Abouelela A., Abbas H.M., Eldeeb H., Wahdan A.A., Nassar S.M., Pattern recognition letters, 26, 10 (2005).
16. Potluri P., Parlak I., Ramgulam R., Sagar T. V., Composites Science and Technology, 66, 2 (2006).
17. Chang C. W., Sud D., Mycek M. A. in "Digital Microscopy – Methods in Cell Biology", 3rd Ed. (G. Sluder, D. E. Wolf, eds.) Vol. 81, pp. 495-524, Academic Press, San Diego, 2007.
18. AATCC Test Method 30-1999. Antifungal Activity, Assessment on Textile Materials: Mildew and Rot Resistance of Textile Materials. 2002. V: AATCC Technical Manual 2002. Research Triangle Park, American Association of Textile Chemists and Colorists, p. 81-84.
19. Stevens D.A., Infectious Diseases in Clinical Practice, 12, 2 (2004).
20. Maertens J.A., Clinical Microbiology & Infection Supplement, 10, 1 (2004).

Received 15.04.2009 Reviewed 10.03.2010