MICROSCOPY INVESTIGATION OF CELLULOLYTIC FUNGI ACTION ON COTTON FIBERS

CARMEN POPESCU, LĂCRĂMIOARA OPRICĂ**, DANIELA PRICOP*, GINA BĂLAN, RODICA MUREŞAN ***, DORINA CREANGĂ***

* Faculty of Physics, "Alexandru Ioan Cuza" University, 11 Carol I Blvd., Iași, Romania,
e-mail: popescu.carmen_mariana@yahoo.com
** Faculty of Biology, "Alexandru Ioan Cuza" University, 11 Carol I Blvd., Iași, Romania,
*** Faculty of Textile and Leather Engineering and Industrial Management,
"Gh. Asachi" University, Iași, Romania

Abstract. In this paper a microscopy study was presented with focus on the cellulolytic fungi action on cotton fibers physical aspect. Qualitative and quantitative analysis was accomplished based on samples of textile material used as support for *Chaetomium globosum* and *Phanerochaete chrysosporium* fungi. Fiber size and mesh aperture changes were evaluated evidencing more invasive attack of *P. chrysosporium* compared to *C. globosum* microorganism. Fiber size changing with 6 to 19% for *C. globosum* was revealed while for *P. chrysosporium* the changes ranged between 20 and 39% and also fibers were broken down. The microscope investigation could be of interest for further study of cotton textile resistance to environmental mildew.

Key words: microorganism, cellulolytic effect, inverted microscope.

INTRODUCTION

The microorganisms that affect cotton are grouped into two large categories: (i) fungi and bacteria that only discolor the fibers, and (ii) fungi and bacteria that actively attack the cellulose and weaken fiber resistance. Also both types of attacks could be developed by some microorganisms [2]. Cotton fiber content in cellulose but also other organic and inorganic substances from its composition make it a suitable substrate for cellulolytic fungi like *Chaetomium globosum* and *Phanerochaete chrysosporium*. Half a century ago for testing the effectiveness of mildew proofing agents on cotton textiles, *C. globosum* Kunze was used as the test organism [1, 6]. This particular fungus contaminating environmental air and water was selected because it was found on nearly all sorts of textiles from cotton. In recent years *Phanerochaete* fungi especially from soil source were used in

Received: March 2014;
in final form May 2014.

biotechnological applications [4] like the conversion of lignocellulose from cotton to valuable products such as ethanol [5]. Modern microscopy devices enable the description of physical aspect changing in cotton fiber textiles following cellulolytic fungi action allowing measurement of fiber and mesh aperture size.

To study physical properties of various textiles microscope techniques are very useful [3, 7]. Inverted microscopes have rapidly gained in popularity since they facilitate examination of living cell culture dishes using standard objectives and avoiding the use of sealed flow chambers – accessories often unavailable and/or expensive. Compared to conventional microscopes working with inverted devices they allow a better access to the stage with investigated specimen. Inverted designs have their center of mass closer to the lab bench and therefore they are less sensitive to vibration. However, there is some risk of physical damage, as objectives may rub against the bottom surface of the stage during rotation of the objective lens turret. Therefore, despite many advantages, inverted research microscopes require more attention than do standard upright designs.

In this paper we describe the results of our experiment with dense cotton textile let for couple of weeks as support for such fungi development.

MATERIALS AND METHODS

OPTICAL INSTRUMENT

Inverted microscope type Nikon Ti Eclipse was used to take images from the analyzed textile samples. Most details of interest were investigated with magnitude orders ranging from 4 to 20×. Specialized soft package Nis Elements Basic Research allowed image processing and measurement with graphical display of size distributions. The images were taken in bright field technique most of them with an objective of 4× magnitude to capture a large scale image while for details of interest the magnitude of the objectives was increased to 20×. Further increasing in the objective magnitude was useless regarding the opacity of the investigated material. The measurements for each type of investigated sample and for each fiber parameter (longitudinal and transversal thickness of fibers as well as mesh aperture area) were performed on four images and the medium value was considered for graphical analysis and discussion.

COTTON SAMPLES

Cotton textile specimens from local industry production were chosen as pieces of 4×4 cm^2. The cotton bought from local commerce is chemically bleached with H_2O_2. To avoid any contamination the specimens were washed by boiling with NaOH and nonionic tenside and then rinsed for many times with warm and cold distilled water.

MICROORGANISMS

Two types of cellulolytic fungi, namely *Chaetomium globosum* and *Phanerochaete chrysosporium* from the collection of Biology Faculty at "Al.I. Cuza" University Iaşi, Romania, were grown on specific agarized culture media – basically malt dextrose Sabouraud medium and respectively peptone glucose Haynes medium. Fungi spore cultures 7 days old were inoculated in Petri dishes containing also specific culture media. Onto the inoculated Petri dishes the specimens of textile material were added and let for incubation in INCUCELL thermostatic room at 28 ºC for couple of weeks. This time duration was chosen according to specific literature [8] where it is stated that for *P. chrysosporium* fermentation on solid substrate, it generally takes more than a week to ensure an appropriate level of enzymes needed for lignin degradation. Cotton textile samples removed from the culture media were carefully manipulated for microscope analysis. Automatic measurement of fiber diameter on two directions, namely "vertical" and "horizontal" as well as mesh aperture area was carried out resulting in tables and histograms.

RESULTS AND DISCUSSION

In the figures below we present some representative images for the microscope investigation of studied textile material from macroscopic scale to 4× and 20× microscope magnitude.

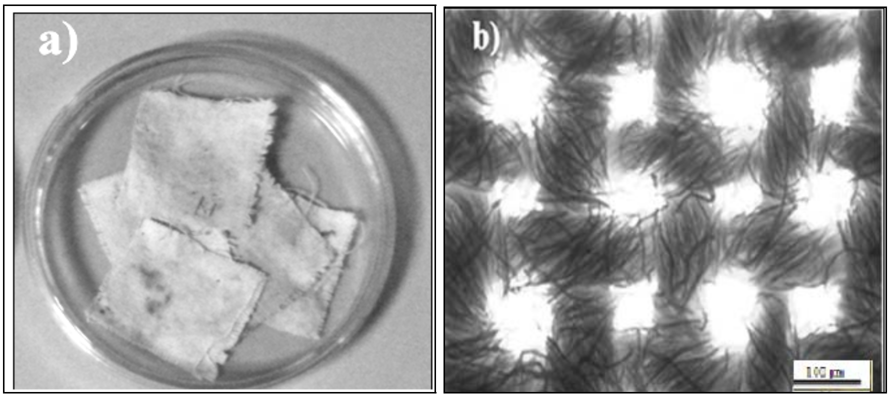

Fig. 1. a) Petri dish with mildew cotton following cellulolytic fungi attack; b) Bright field image of reference cotton sample before fungi attack (4× objective).

In Fig. 1 a, the mildew cotton textile specimens could be seen at macroscopic scale, the fungi attack being observable with naked eye in the form of dark spots. The reference textile sample analyzed by inverted microscope before fungi action in Fig. 1 b is presented in the form of a specific image provided in bright field technique. In Fig. 2 a, b the effect of *C. globosum* cellulolytic action can be seen – with modified physical aspect of the textile appearance (fiber mesh distortion, Fig. 2 a) and also with fungus cell agglomeration (Fig. 2 a, b). The estimated extension of fungi accumulation areas (Fig. 2 b) ranged from 14790 μm^2 to 5730 μm^2. After *Chaetomium* fungi cultivation important changes could be observed in vertical and horizontal fiber diameter histograms (Fig. 4 and Table 1).

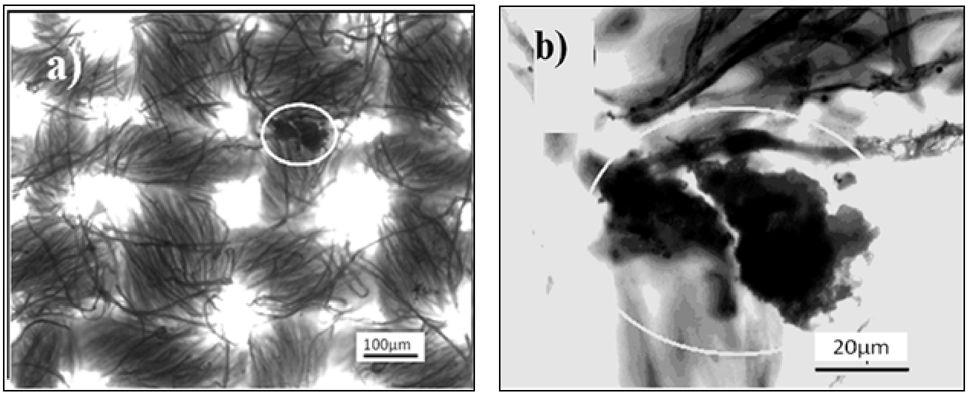

Fig. 2. Bright field images: a) Cotton/*C. globosum/* 4× objective; b) Cotton/*C. globosum* – detailed image of fungi accumulations (20× objective).

In Fig. 3 the histograms of fiber dimensions in reference cotton textile sample are shown. Relatively narrow range of fiber diameter size was evidenced; for the vertical fibers the thickness was ranging from 80 μm to 150 μm (Fig. 3 a) while the size of horizontal fibers ranged from 80 μm to 130 μm (Fig. 3 b).

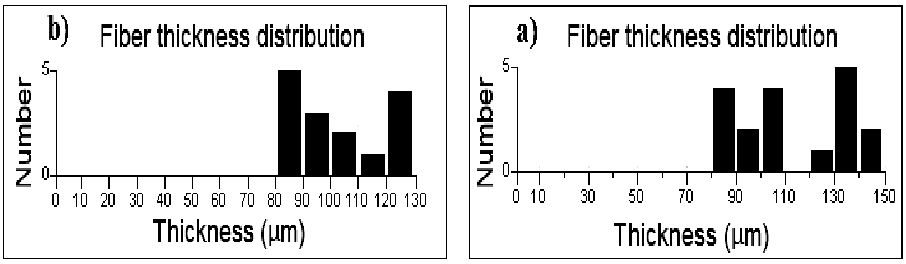

Fig. 3. Thickness distribution of fibers in simple cotton: a) vertical fibers; b) horizontal fibers.

The average values presented in Table 1 show an increase of 6% and respectively 17% for the two measurement directions in the case of *C. globosum* fungi. Mesh aperture was consequently diminished with about 36% in average. Fiber thickness distribution histograms show the presence of enlarged fibers – up to

140 µm (Fig. 4 a) and respectively up to 200 µm (Fig. 4 b). Also a few number of cotton fibers with smaller thickness compared to the reference sample were noticed – of about 65 µm – Fig. 4 a.

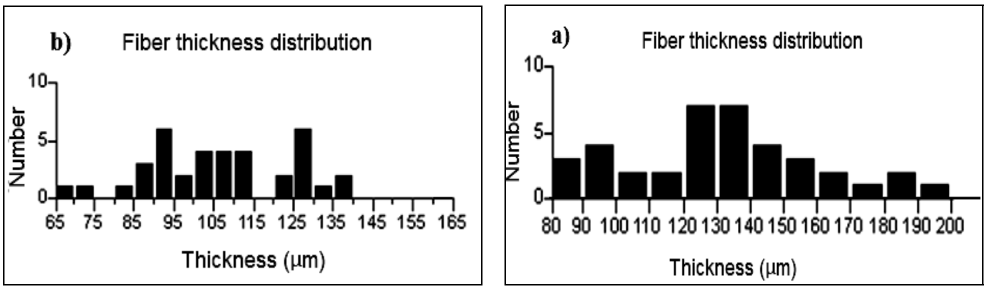

Fig. 4. Thickness distribution of fibers in cotton/*C. globosum*; a) vertical fibers; b) horizontal fibers.

In Fig. 5 the effect of *P. chrysosporium* fungus is illustrated. A stronger attack than in the case of previous cellulolytic fungus was evidenced with many fibers broken down (Fig. 5 a) and with considerably frequent fungi accumulation areas (Fig. 5 b).

Table 1

Mean sizes of cotton fibers

Sample	Average length of vertical fibers (µm)	Average length of horizontal fibers (µm)	Average mesh aperture area (µm^2)
Cotton	112.7	107.6	5666.1
Cotton/*Chaetomium globosum*	132.0	114.1	3589.4
Cotton/*Phanerochaete chrysosporium*	89.9	64.7	9780.5

For fibers that kept integrity the microscopic measurements were done resulting in histograms shown in Fig. 6 and average values listed in Table 1.

According to Table 1 the average values of fiber diameters were reduced with 20% to 39% that made the aperture area increase with over 70%. Fungi accumulation area ranged from 2080 µm^2 to 3600 µm^2 (Fig. 5 b). The histograms in Fig. 6 present a significant number of fibers with diameters as small as 10 to 70 µm – never found in reference cotton textile sample.

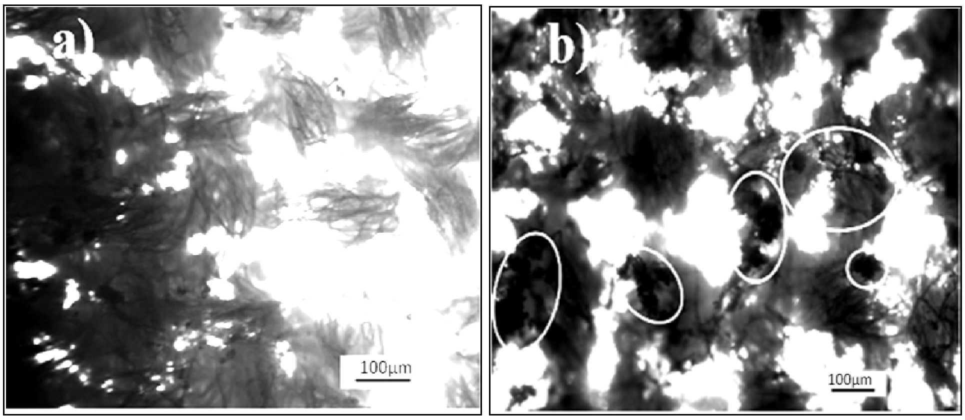

Fig. 5. Image of a) Cotton/ *P. chrysosporium/4×* objective; b) Cotton/ *P. chrysosporium* with fungi.

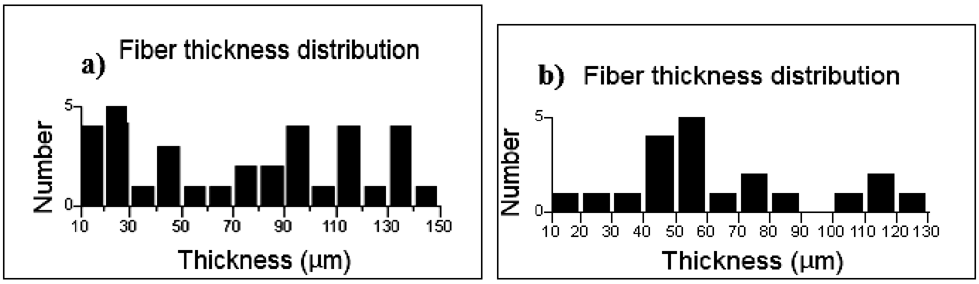

Fig. 6. Thickness distribution of fibers on cotton/*P. chrysosporium* a) vertical fibers; b) horizontal fibers.

From the biotechnological point of view the more aggressive action of *P. chrysosporium* on cotton material suggests that a further research step intended for textile chemical treatment against environmental agents should be focused on the cotton protection against this fungus. Also in future investigation another cellulolytic fungus will be considered – *Trichoderma viridis*. Comparative data and discussion could be of interest for textile industry yielding resistant materials for campus and military activities.

CONCLUSION

The cellulolytic fungi *C. globosum* frequently found in open air atmosphere proved to have a moderate effect on the industrial cotton textile its action consisting in fiber size diminution and mesh aperture area increase – which make the material more permissive for water and particles from the environment. The enzymatic attack of *P. chrysosporium* was found of highest concern because fibers were partially destroyed meaning that textile protection against this microorganism is imperiously needed especially in case of cotton material for objects to be used in contact with the wet soil – like in camping activities.

REFERENCES

1. ABRAMIUC, D., *Cercetări privind funcționalizarea materialelor textile prin utilizare de compuşi biologic activi*, Ph.D thesis, Faculty of Textiles – Leather and Industrial Management, “Gheorghe Asachi” Technical University from Iaşi, 2013.
2. KADRMOIDHEEN, M., P. SARANRAJ, D. STELLA, Effect of cellulolytic fungi on the degradation of cellulosic agricultural wastes, *Int. J. Appl. Microbiol. Sci*. 2012, **1**(2), 13–23.
3. LONGONI, P., M. RODOLFI, L. PANTALEONI, E. DORIA, L. CONCIA, A. PICCO, R. CELLA, Functional analysis of the degradation of cellulosic substrates by a *Chaetomium globosum* endophytic isolate, *Appl. Environ. Microbiol*., 2012, **78**(10), 3693–3705.
4. MURPHY, D., *Fundamentals of light microscopy and electronic imaging*, Wiley-Liss, New York, 2001.
5. SHI, J., M.S. CHINN, R.R. SHARMA-SHIVAPPA, Microbial pretreatment of cotton stalks by solid state cultivation of *Phanerochaete chrysosporium, Bioresour. Technol*., 2008, **99**(14), 6556–6564.
6. SHI, J., R.R. SHARMA-SHIVAPPA, M.S. CHINN, Microbial pretreatment of cotton stalks by submerged cultivation of *Phanerochaete chrysosporium*. *Bioresour. Technol*., 2009, **100**(19), 4388–4395.
7. SOYTONG, K., S., KANOKMEDHAKUF, V., KUKONGVIRIYAPA, M., ISOBE, Application of *Chaetomium* species (Ketomium) as a new broad spectrum biological fungicide for plant disease control: A review article, *Fungal Divers*., 2001, **7**, 1–15.
8. SUZUKI, Y., N. SAKAI, A. YOSHIDA, Y. UEKUSA, A. YAGI, Y. IMAOKA, S. ITO, K. KARAKI, K. TAKEYASU, High-speed atomic force microscopy combined with inverted optical microscopy for studying cellular events, *Nature, Sci. Rep*., 2013, **3**, 2131.
9. ZHANG, J., X. REN, W. CHEN, J. BAO, Biological pretreatment of corn stover by solid state fermentation of *Phanerochaete chrysosporium*, *Front. Chem. Sci. Eng.*, 2012, **6**(2), 146–151.