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Microbial Degradation of Woven Fabrics and Protection Against Biodegradation

Beata Gutarowska[1] and Andrzej Michalski[2]
[1]*Technical University of Lodz, Institute of Technology Fermentation and Microbiology, Lodz,*
[2]*Spółdzielnia Inwalidów ZGODA, Konstantynów Łódzki, Poland*

1. Introduction

The textile industry is one of the most important and fastest developing industries in the world. An significant problem encountered by manufacturers is that of ensuring that the fabrics produced are of suitable quality and durability. Particular attention needs to be paid to the destructive action of microorganisms present in the environment.

In favourable conditions these can rapidly destroy material, rendering it entirely unusable and causing substantial economic losses.

In 1960, in the UK, annual losses due to biological degradation of cotton fibres were put at 110 000 tonnes of cotton, which at time was 1% of output (Howard & Mc Cord). According to estimates by Hueck-van der Plas (1971), the process of biodeterioration affected 2% of annual production of natural and artificial fibres (Zyska, 1977).

At the start of the 21st century annual world consumption of unwoven fabrics (for practical and technical uses) stood at 46 million tonnes, of which synthetics and cotton accounted for 49% and 42% respectively (with an upward trend in subsequent years), wool for 5%, and other fabrics 4% (linen, sisal, silk and others, with a downward trend) (Central Statistical Office Yearbooks – *Roczniki GUS*, Poland 2008). If 2% of the global value of fibre production is assumed, the problem of microbiological decomposition may affect 920 000 tonnes of fabric annually.

Not all losses can have a price attached to them: museum fabrics are particularly rapidly damaged by microorganisms, and the artistic and cultural value of these items cannot be recreated.

Microorganisms which attack textile products not only have a destructive effect, but also pose a significant danger to human health. Particularly dangerous are the pathogenic microorganisms present on fabrics which come into direct contact with the human body, such as on dressings and surgical masks; this may lead to skin infection, and even heart disorders and pneumonia.

It is a significant challenge for manufacturers to produce fabrics with antimicrobial properties – namely bioactive fabrics, containing biocides to provide protection against pathogenic microorganisms.

A separate issue is the protection of finished textile materials against biodegradation through proper storage, and possibly the use of an appropriate process of disinfection which can effectively eliminate microorganisms without affecting the material's strength properties.

2. Microbial degradation of fibers

Textiles are easily attacked by microorganisms, which means that they quickly become damaged. Microorganisms pose a threat to textile materials at all stages of their production – from the obtaining of raw material (for example on plantations), through to the transportation and storage of the raw material and of the finished product.

Microbial degradation of fabrics depends primarily on their chemical composition. Fabrics of natural origin are particularly susceptible to attack by microorganisms.

The decomposition of natural plant-based fibres caused by the presence of fungi was known as early as 1926–1928, and was described by Smith and Morris. Research into the mechanism of the decomposition of such fibres by microorganisms has continued for 80 years (Zyska, 1977). The main component of plant fibres is **cellulose**. The content of cellulose depends on the type of fibre – in cotton it reaches 94%, in linen fabric around 80%, and in others from 63% to 77% (jute, sisal, hemp). Cellulose is a polysaccharide composed of molecules of β-glucose linked by 1,4-β-glycoside bonds. The number of glucose molecules in a chain ranges from 7 to 10 thousand. Chains may be arranged in parallel, forming a crystalline structure, or tangled to form an amorphous structure. Cellulose is broken down by microorganisms through a process of enzymatic hydrolysis. This mechanism involves a multistage decomposition of cellulose to glucose, brought about successively by the enzymes 1,4-endo-β-D-glucan cellobiohydrolase (EC 3.2.1.91) (also called exoglucanase, cellobiohydrolase), endo-1-4-β-D-glucan glucanohydrolase (EC 3.2.1.4) (endoglucanase, β-glucanase) and glucohydrolase of β-D-glucosides (EC 3.2.1.21) (cellobiose, β-glucosidase) (Evans, 1996; Jeffries, 1987; Szostak-Kot, 2005).

The intensity of cellulose decomposition is indicated by the appearance of differently coloured stains on fabrics (carotenes, anthraquinones, excreted by the microorganisms), reduction in the degree of polymerization, breakage of the fibre structure and reduction in tearing strength. In extreme cases the cellulose may decompose completely.

Plant fibres also contain small quantities (up to 10%) of such compounds as hemicellulose and lignin, which give the fibres rigidity, and pectins, which act as a kind of glue. Many microorganisms are capable of producing enzymes which decompose hemicelluloses and pectins (xylanase, galactosidase, mannosidase, glucuronidase, pectinesterase, glycosidase and others) (Bujak & Targoński, 1990; Szostak-Kot, 2005). Lignin is the least rapidly decomposed component of plants, because of its structure – phenylpropane compounds are linked by ether and carbon bonds and are very resistant to enzymatic decomposition. In spite of this there are certain species of fungi and bacteria which are capable of decomposing lignin (*Chaetomium, Paeciliomyces, Fusarium, Nocardia, Streptomyces, Pseudomonas, Arthrobacter* and others) (Szostak-Kot, 2005; Targoński & Bujak 1991).

The rate of decomposition of natural plant-based fibres depends on their chemical composition. Among cellulose-based fibres, the slowest to decompose is jute (35% non-cellulose substances, including 25% lignin) (Basu & Ghose, 1962; Szostak-Kot, 2005). The rate also depends on many other factors: apart from environmental factors and the type of microorganisms, there is also an effect from thickness, type of weave, degree of crystallinity (amorphous cellulose is more easily degraded) and degree of orientation (namely the angle made by the fibrils with the long axis of the fibre – highly oriented fibres are less susceptible to biodeterioration) (Pedersen et al., 1992; Salerno-Kochan & Szostak-Kotowa, 2001; Szostak-Kot, 2005; Tyndal, 1992).

Artificial cellulose fibres include regenerated fibres (rayon) and cellulose acetate. Rayon usually has a lower degree of crystallinity, polymerization and ordering than cotton. It is also highly hygroscopic (its capacity to absorb water in normal conditions is 9.8–13%), which is a reason for its common use in making woven and knitted fabrics and as an additive to natural and synthetic fibre products. Its rate of microbiological decomposition is comparable to that of cotton. Cellulose acetate is produced by the acetylation of cellulose with acetic anhydride, as a result of which the product has a maximal degree of acetylation, and the fibre becomes more resistant to microbiological decomposition than cellulose (Buchanan et al., 1993, Buschle-Diller et al., 1994; Salerno-Kochan & Szostak-Kotowa, 2001; Szostak-Kot, 2005).

Wool is characterized by high strength, thermal insulation properties and hygroscopicity (it can absorb 50% moisture without feeling wet). Chemically, wool is built from three types of keratins: low-sulphur, high-sulphur and high-tyrosine. Low-sulphur keratins primarily are linked with each other and to proteins of the matrix by numerous bonds – sulphide bridges, covalent bonds and hydrogen bonds, and in the presence of water also hydrophobic bonds. Due to the presence of these bonds and the network structure of wool, it is resistant to stretching and tearing and to environmental factors, including enzymatic degradation.

The biodeterioration of woollen fabrics involves microorganisms with mainly proteolytic and keratinolytic enzymes. So far 299 species of fungus with keratinolytic properties have been described, of which 107 are pathogenic to humans (Błyskal, 2009). Decomposition of a woollen fabric proceeds by way of deamination, sulphitolysis and proteolysis (Kunert, 1992, 2000). The first stage involves the splitting of disulphide bridges, which are the source of keratin's resistant strength. This is followed by the enzymatic decomposition of proteins by proteolytic enzymes (proteases) into oligopeptides, and these are then broken down by peptidases into amino acids, which are used in metabolic processes of oxidative deamination with the release of ammonia (Gochel et al., 1992; Kunert, 1989; Szostak-Kot, 2005). Characteristic symptoms of the microbiological decomposition of wool include the variously coloured stains on the fabric surface, a distinctive smell (in anaerobic conditions H_2S is produced), and loss of stretching strength.

During the technological process the woollen raw material is subjected to mechanical, chemical and photochemical action, which increases the susceptibility of the fibres to biodegradation. Many problems have been reported and described resulting from the development of microorganisms on woollen textiles, for example when carpets are in storage (Gochel et al., 1992; Hoare, 1968; Simpson, 1987). In favourable conditions of temperature (37°C) and humidity of the material (25–75%), the number of fungi may increase to as much as 109 CFU/1g of wool over 20 days (Zyska, 2001).

Natural **silk** is a fibre produced from the cocoon shell of the mulberry silkworm. Silk is characterized by high strength, elasticity, thermal insulation properties and hygroscopicity (in natural conditions silk contains approximately 11% moisture, and it can have a moisture content of 30% without feeling wet).

Raw silk consists of protein fibres – fibroins – stuck together with the protein sericin. The chains are linked by disulphide bridges, which give the fibre its strength; there are also hydrogen bonds within and between molecules. This polypeptide has a crystalline structure, and around 90% of it consists of four amino acids: alanine, glycine, serine and tyrosine. Textile manufacturers formerly used raw silk, which was resistant to the damaging action of light (chiefly ultraviolet) and was stronger, although the fabric yellowed with time. Fabrics are now made from degummed silk (with the sericin removed) – this material does not yellow under the action of light, and is more resistant to microbiological decomposition (Becker et al., 1995; Kaplan et al., 1994; Szostak-Kot, 2005). Microorganisms probably assimilate sericin more easily than fibroin. The decomposition of sericin involves mainly proteolytic enzymes of microorganisms (Forlani et al., 2000). In vitro tests have also confirmed the degradation of fibroin by protease (Horan et al., 2005).

Synthetic fibres are obtained by means of polymerization. The most commonly used types are polyamide, polyester, polyurethane and polyacrylonitrile fabrics. Synthetic fabrics are resistant to biodeterioration as a rule, and if the process occurs, it is a long-lasting one.

Synthetic fibres which have undergone a process of biodegradation become less resistant to stretching (by as much as 20–30%), undergo swelling (increase in diameter by up to 20%), and change colour due to microbially produced dyes and acidic products which react with the dyes present in the fabrics (Zyska, 2001)..

Mechanisms of biodegradation involve physical damage to fibres and chemical decomposition due to numerous metabolites produced by microorganisms (ammonia, nitrates, hydrogen sulphide, organic acids) or by an enzymatic route (activity of lipases, esterases, proteases, ureases) (Lucas et al., 2008).

Polyamide fibres contain amide groups in the main chain of their macromolecules. Greatest interest is shown in aliphatic polyamides, and among them, polyamide 6 (Steelon, Perlon) and polyamide 6.6 (Nylon). Polyamides are resistant to microbiological decomposition, although research is carried out using various strains of microorganisms which contribute to that process. It has been found that some bacterial and fungal oxidases and hydrolases (for example manganase peroxidase from white rot Basidiomycetes) decompose aliphatic polyamides, leading to their depolymerisation (Friedrich et al., 2007; Lucas et al., 2008).

In the textile industry there are two types of **polyurethane** fibres used: high-crystalline types with a linear structure, and highly elastic segmental fibres of the Spandex type. High-crystalline fibres have a similar structure to polyamides, and display high rigidity. The highly elastic type of fibres contain a minimum of 85% polyurethane polymer with a segmental structure. This fibre has a very large extension at rupture, colour permanence, and resistance to radiation and ageing. Microbial degradation of polyurethanes occurs by way of chemical hydrolysis, as a result of the extracellular action of esterase enzymes (Akutsu et al., 1998; Allen et al., 1990; Ruiz et al., 1999).

Polyester fibres are produced from large-molecule compounds with repeating ester bonds in the main chain. The type most commonly used in the textile industry is poly(ethyl terephthalate) (PET), while among aliphatic polyesters polylactic acid (PLA) is beginning to take on great importance. Polyester fibres containing terephthalate are resistant to microbiological decomposition, although in research into the effect of soil microflora such fabric displayed changes in fibre structure, which may indicate the possibility of biodegradation over a long period (Salerno-Kochan & Szostak-Kotowa, 1997). The processes of decomposition of PLA may involve enzymes such as proteinase K (Li & Vert, 1995).

Polyacrylonitrile fibres are produced from polyacrylonitrile, or else are copolymers of acrylonitrile with other monomers containing groups capable of reacting with reactive dyes. These fibres have high resistance to atmospheric effects, a pleasant feel, good strength and resistance to chemical and biological agents. Polyacrylonitrile, as well as dipolymers and terpolymers of acrylonitrile, are resistant to microbiological decomposition, although at high air relative humidity (90%) mould attack on the surface of polyacrylonitrile has been described (Zyska, 2001).

Biodeterioration of fabrics is mainly caused by filamentous fungi, and to a lesser extent by bacteria. Microorganisms capable of degrading natural and artificial fibres are listed in Table 1. (based on a survey of the literature).

3. Conditions favourable for biodegradation of fibres and fabrics

The rate of microbiological decomposition of fabrics is affected by environmental factors such as air relative humidity, temperature, light, and the properties of the fabrics, chiefly their chemical composition, fibre structure, density and thickness of weave, and the type of substances used in the finishing of the unwoven fabric (Szostak-Kot, 2005).

High humidity in a fabric is the most important factor affecting the development of microorganisms. The absorption of water by a fabric depends, among other things, on its hygroscopicity and porosity. A level of fabric relative humidity above 65% increases fibre swelling and favours the development of microorganisms, particularly moulds, on the fabric. The development of bacteria requires a high fabric relative humidity, above 95% (Szostak-Kot, 2009). At the **temperature** used for fabric storage (20–35°C) many microorganisms develop on the fabrics, and the range within which microorganisms develop is significantly greater (4–50°C, excluding extremophilic microorganisms).

All fibres are sensitive to photo-oxidation caused by **light radiation** (particularly ultraviolet and infrared). Ultraviolet radiation in cellulose fibres, such as cotton, causes breakage of the cellulose chain and leads to its decomposition. Wool and silk are also susceptible to photochemical degradation, particularly in the presence of oxygen – for example the photodegradation of fibroin in silk occurs as a result of the breakage of hydrogen bonds and oxidation of tyrosine. Biodegradation of silk may be favoured by prior photodegradation under the action of ultraviolet (Sionkowska & Planecka, 2011). The action of infrared radiation on textile material causes overheating of the surface and leads to many physicochemical changes. Light, increased temperature and atmospheric impurities additionally speed up the process of ageing, and in such conditions fabrics may also be more sensitive to attack by microorganisms (Szostak-Kot, 2009).

Physical features of fabrics, such as fabric thickness and density of weave, may enable the spread of microorganisms and processes of fabric destruction (thinner fabrics with a looser weave are subject to more rapid decomposition). The microbiological decomposition of a fabric is also affected by the substances added to the fabric, such as dyes, glues and treatments. These may provide an additional source of food for microorganisms, or else may have a negative effect on their development (Szostak-Kot, 2005). Many substances currently used in the textile industry are characterized in terms of susceptibility to microbiological decomposition or effect on microorganisms.

4. Protection of fibres against microbial degradation

Control of environmental conditions during storage, transportation and use is an effective method for protecting fibres against biodeterioration. This involves the maintenance of constant environmental conditions which are unfavourable to microorganisms, with the use of ventilation and air-conditioning devices. The temperature in storage rooms should be maintained at 18–20°C, and the air relative humidity should not exceed 60% (Szostak-Kot, 2005).

To combat the development of microorganisms, chemical compounds known as **biocides** are used. These are added at various stages of fibre production. Biocides make it possible to eliminate microorganisms effectively, but if used improperly may cause damage to the health of the user.

Modern biocides are expected to satisfy several basic criteria (Figure 1): high effectiveness at low concentrations (of the order of ppm) against a wide spectrum of microorganisms, absence of increased immunity of microorganisms, lack of effect on the properties of the fabric, absence of toxic or allergenic action or irritant action to the skin and mucous membranes in humans and animals, high biodegradability following application, good water solvency, low volatility, absence of smell, high stability (durability), absence of corrosive action on technical materials, and favourable price.

Nonvowens	Microorganisms isolated from nonwovens and/or able to biodegradation of nonwoven	Author , year
Cotton	**Fungi:** *Aspergillus* sp. *(A.versicolor, A.flavus, A.fumigatus, A.niger, A.terreus, A.nidulans, A.ustus, A.fischerii, A.flaschentraegeri);Penicillium* sp. *(P.notatum, P.citrinum, P.funiculosum, P.cyclopium, P.janthinellum); Cladosporium* sp. *(C.macrocarpium, C.herbarum); Cheatomimum* sp. *(Ch.globusum, Ch.cochlioides); Alternaria* sp. *(A.tennuis, A.geophila); Trichoderma* sp. *(T.viride, T.reesei); Fusarium nivale; Myrothecium* sp.; *Memnoniella* sp.; *Stachybotrys* sp.; *Verticillum* sp.; **Bacteria:** *Cytophaga* sp.; *Cellulomonas* sp.; *Bacillus* sp.; *Clostridium* sp.; *Sporocytophaga* sp.; *Microbispora bispora*	Abdel-Kareem et al., 1997; Bartley et al., 1984; Evans, 1996; Flannigan et al., 2001;Kowalik, 1980; Kubicek et al.,1988

Nonvowens	Microorganisms isolated from nonwovens and/or able to biodegradation of nonwoven	Author , year
Flax	**Fungi:** *Aspergillus* sp. *(A.flavus, A.fumigatus, A.niger, A.terreus, A.nidulans, A.ustus, A.fischeri, A.auratus, A.carbonarius, A.proliferans, A.spinulosus); Penicillium* sp. *P.funiculosum,P.rajstrickii, P.biforme, P.soopi) Trichoderma viride; Alternaria alternata; Cheatomium cochlioides; Fusarium nivale*	Abdel-Kareem et al., 1997
Wool	**Fungi:** *Aspergillus* sp. (*A. cervinus, A. fischeri, A.flavus, A. fumigatus, A.nidulans, A.niger, A.rapier, A.sparsus, A.spinulosus, A.ventii); Chrysosporium* sp.; *Penicillium* sp. (*P.canescens, P.cyclopium, P.granulatum, P.lanoso, P.paxilli, P.soopi); Microsporum* sp.; *Trichopchyton* sp.; *Fusarium* sp.; *Rhizopus* sp.; *Cheatomium* sp.; *Alternaria* sp.;*Ulocladium* sp.; *Stachybotrys chartarum; Scopulariopsis brevicaulis; Acremonium* sp.; **Bacteria:** *Bacillus* sp. *(B.mesentericus, B. subtilis, B.cereus, B..mycoides); Pseudomonas* sp.; *Streptomyces* sp. *(S.fradiae)*	Abdel-Kareem et al., 1997; Abdel-Gawada, 1997; Agarwal & Puvathingal, 1969; Błyskal, 2009; Kowalik, 1980; Lewis, 1981; McCarthy & Greaves, 1988; Nigam & Kushwaha, 1992; Safranek & Goos, 1982
Silk	**Fungi:** *Aspergillus* sp. (*A.flavus, A.niger, A.rapei); Penicillium* sp. (*P.canescens,P.paxilli*); *Chaetomium* sp.; *Cladosporium* sp.; *Rhizopus* sp.; **Bacteria:** *Bacillus megaterium; Pseudomomas* sp. (*P.aureofaciens, P.chlororaphis, P.paucimobilis P.cepacia); Serratia* sp.; *Streptomyces* sp.; *Variovorax paradoxus*	Abdel-Kareem et al., 1997; Forlani et al., 2000; Ishiguro & Miyashita, 1996; Nigam et al., 1972; Sato, 1976; Seves et al., 1998
Polyamide	**Fungi:** *Aspergillus* sp. (*A.niger); Penicillium* sp. *(P.janthinellum); Blennoria* sp.; *Monascus* sp.; *Tritirachium oryzae; Absidia* sp.; *Trichosporon* sp.; *Rhodotorula* sp.; white rot *Basidiomycetes* **Bacteria:** *Pseudomonas* sp. *(P.aeruginosa); Protaminobacter* sp.; *Achromobacte* sp.; *Brevibacterium* sp.; *Flavobacterium* sp.; *Alcaligenes* sp.; *Bacillus* sp. (*B.pallidus); Corynebacterium* sp.	Bailey et al., 1976; ; Cain, 1992; Denizel et al.,1974; Ennis et al., 1978; Nigam et al., 1972; Prijambada et al., 1995; Szostak-Kotowa, 2004; 2005
Polyurethane	**Fungi:** *Aspergillus terreus; Penicillium* sp.; *Cladosporium* sp.; *Paecilomyces* sp.; *Alternaria* sp.; *Trichoderma* sp.; *Stachybotrys* sp.; *Chaetomium globusom; Curvularia senegalensis; Fusarium solani; Aureobasidium pullulans; Glicoladium roseum; Stemphylium* sp. **Bacteria:** *Pseudomonas* sp.; *Acinetobacter calcoaceticus; Arthrobacter globiformis*	Halim El-Sayed et al., 1996; Howard, 2002; Szostak-Kotowa, 2004; Wales & Sagar, 1988
Polyacrylo-nitrile	**Fungi:** *Aspergillus* sp.; *Penicillium* sp.; *Stachybotrys* sp. **Bacteria:** *Arthrobacter* sp.	Szostak-Kotowa, 2004; Yamada et al., 1979; Zyska, 2001

Table 1. Fibre-degrading microorganisms

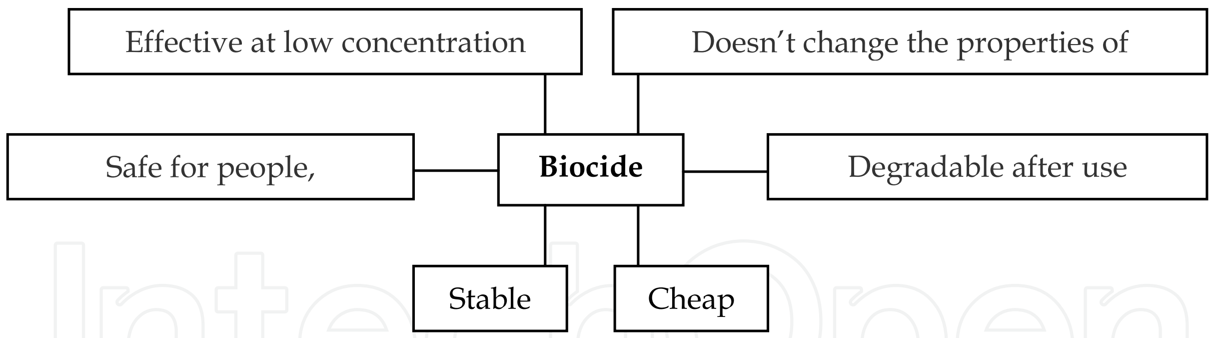

Fig 1. Features of good biocide for nonwovens

There are few active compounds that meet all of these requirements, and therefore work is still being done to find substances for use in fabrics with the desired properties.

5. Biocides approved by the EU for use in the textile industry

Biocides which can be used in the textile industry belong to biocidal compounds category II and to group 9 on the list of biocidal compounds (under Directive 98/8/EC), and include 134 active substances. The active substances in category II which can be used in the textile industry belong to eight groups of chemical compounds: inorganic compounds, compounds of nitrogen, phenol and their derivatives, compounds of halogens and their derivatives, oxidizing compounds, alcohols, aldehydes, organic acids and their derivatives (Table 2).

Mechanisms of action on microorganisms depend on the type of compound, and often take multiple routes. Biocides cause disturbance of the functioning of the cytoplasmic membrane and cell wall, inactivation of proteins, slowing of DNA synthesis, and many other types of damage to the cells of microorganisms (Brycki, 2003).

Examples of commercial preparations containing the listed chemical compounds, which are currently used frequently in the textile industry, are listed in Table 3. On the international market many firms also offer ready-made fibres with antimicrobial properties, containing biologically active substances – examples of these are given in Table 4.

However, the mass use of chemical preparations and ready-made fibres containing biologically active substances lead to an increase in microorganisms' resistance to biocides. There are also increased requirements in terms of high effectiveness against a wide spectrum of microorganisms. For this reason new solutions are constantly being sought – new compounds or mixtures of compounds, and methods for stabilizing them and applying them to fabrics. The development of new technologies related to the production of fibres with antimicrobial action has proceeded particularly rapidly in the last decade. An overview of selected research into production and antimicrobial activity of natural and artificial fibres containing biocides is presented in Table 5. Extensive data concerning polymeric materials can be found in the survey paper by Munoz -Bonilla and Fernandez- Garcia (2011).

Among the biocides used in both natural and artificial fibres, there is a high level of interest in quaternary ammonium salts and phosphonium salts. The role of biological agent is played by the fibre additive chitosan, as well as antibiotics tetracycline, cephalosporin, vinyloimidazol, ciprofloxacin, and antifungal clotrimazol, ketokonazol. There has recently also been great interest in inorganic nanoparticles as agents with antimicrobial properties.

The obtaining of nanoparticles of metals or nanostructured fibres has, thanks to the increase in surface area, led to the achievement of new characteristics desirable in the textile industry, and significantly greater effectiveness in destroying microorganisms. High activity have: TiO_2 nanoparticles, metallic and non metallic TiO_2 nanocomposites, titania nanotubes (TNTs), silver nanoparticles, silver-based nanostructured materiale, gold nanoparticles, zinc oxide nanoparticles and nano-rods, copper nanoparticles, metallic and inorganic dendrimers nanocomposite, nanocapsules cyclodextrins containing nanoparticles. New methods of obtaining such fibres with the addition of nanoparticles are constantly being developed, and the stabilization of nanoparticles on the surface of fibres is also of great importance (Dastjerdii et al., 2009; Dastjerdi & Montazer, 2010, Silver, 2003).

One of the conditions which a biocide is required to satisfy is its safety, and therefore much attention is currently being paid to substances of natural origin which are not toxic or allergic and are easily biodegradable. Some natural dyes and substances extracted from plant seeds and fruit contain active substances which slow the development of microorganisms and can be used to produce biologically active fabrics (Table 6).

The active substances	Antimicrobial activity	Mechanisms of antimicrobial activity
Inorganic compounds metals such as silver, zinc, copper, metal oxides such as titanium dioxide, metal salts	gram positive, gram negative bacteria, fungi, viruses	Inhibition of DNA replication, denaturation of proteins, abnormal functioning of the cytoplasmic membrane, outflow of the low molecular masses intracellular components from cell, disruption of transport of electrons and protons
Nitrogen compounds aliphatic amines such as N-(3-aminopropyl)-N-dodecylopropano-1 ,3-diamine; bis (3-aminopeopylo) octylamine; quaternary alkyl ammonium salts such as chloride, didecyl dimethyl ammonium chloride, alkylobenzylodimetyloamomonium chloride; guanidine, alkyl of aza compounds, oksaaza, tiaaza aromatic compounds	gram positive, gram negative bacteria, fungi, viruses	Damage and dysfunction of cytoplasmic membrane and cell wall, outflow of the low molecular masses intracellular components from cell, guanidine - inhibition of DNA replication, protein denaturation
Phenol and its compounds mono cyclic compounds such as chlorocresol, chloroxylenol, bis-phenol compounds, triclosan, dichlorophen, biphenyl-2-ol	gram positive, bacteria (including *Mycobacterium tuberculosis*) , gram negative bacteria, viruses	Inhibition of DNA replication, denaturation of proteins, damage and dysfunction of cytoplasmic membrane and cell wall, outflow of the low molecular masses intracellular components from cell
Halogens and their compounds inorganic: chlorine, iodine, sodium	gram positive, gram negative	Denaturation of proteins, damage and dysfunction of cytoplasmic

The active substances	Antimicrobial activity	Mechanisms of antimicrobial activity
and calcium hypochloride, sodium chlorate, chlorine dioxide; organic: chloroarylamides, halohydantoin, chloroisocyanuric acid	bacteria	membrane and cell wall, outflow of the low molecular masses intracellular components from cell
Oxidizing compounds peracetic acid, , peroxyoctanoic acid, hydrogen peroxide, 2-butanone peroxide	gram positive, gram negative bacteria, viruses	Transformation of sulfhydryl groups to di sulfide bridges in protein - protein deactivation
Alcohols propan-2-ol, 2-phenoxyethanol, benzyloxymethanol, 2,4-dichlorobenzyl alcohol-	gram positive, gram negative bacteria	Inhibition of DNA replication, denaturation of proteins
Aldehydes formaldehyde, dialdehydes glyoxal, glutaraldehyde, orthophthalic aldehyde	gram positive (including *Mycobacterium tuberculosis,* and bacterial spores), gram negative bacteria, viruses,	Inhibition of DNA replication, denaturation of proteins (by joining the amino groups), dysfunction of cytoplasmic membrane and cell wall, outflow of the low molecular masses intracellular components from cell
Organic acids and their compounds aliphatic: carboxylic acids: formic, glycolic, lactic, nonanoic; aromatic acids: benzoic, salicylic karbaminic	gram positive, gram negative bacteria	Inhibition of DNA replication, denaturation of proteins, damage and dysfunction of cytoplasmic membrane and cell wall, outflow of the low molecular masses intracellular components from cell

Table 2. Biocides used in protection of nonwovens and their antimicrobial activity (based on: Brycki, 2003)

Chemical preparations (trade name)	The active substances
Afrotin ZNK 10 & ZNL	Zinc pyridinate
Actifresh RT-87-11	Alkaline mixture of halogenated organic compounds
Armesan A	Phenyloxy-chloro-phenol
Biocide PB 940	2,2 '-dihydroxy-5 ,5-dichloro-diphenyl-mono-sulfide
Cuniculate 2419-75;Mystox 8	8- copper quinolinate
Densil P	Dithio-2, 2'-biobenzomethylamide
Esterol 100 CD; Mystox LPL	Pentachlorophenol laurate
Fungitex ROP Dichlorophen	Bis (chlorohydroxyphenyl) methane
GivGard DXN	6-acetoxy-2 ,4-dimethyl-1 ,3-dioxane
Kathon LM	2-octyl-4-isotiazolino-3-one
Metanit 55-61	Carbendazim + diuron
Myacide	2-bromo-2-nitropropane-1 ,3-diol
Myacide SP	2,4-dichloro-benzyl alcohol

Chemical preparations (trade name)	The active substances
Nuodex zinc naphtenate	Zinc naphthenate
Nuodex copper naphtenate	Copper naphtenate
Preventol GD	2,2 '-dihydroxy-5, 5'-dichloro-diphenylmethane
Preventol O extra	2-hydroxy-biphenyl
Preventol R80 & R50	Quaternary ammonium salts
Sanitized BSC	Tiobendazol
Sanitized DET 8530	Quaternary ammonium salts
Tolcide C30	2'-(thiocyanomethylthio) benzothiazole

Table 3. Chemical preparations with antimicrobial activity used in the textile industry (based on: McCarthy, 1995; Evans, 1996)

Bioactive nonwovens/ producer	Antimicrobial properties of nonwovens
Navaron/ Taogoesei Chemical Industry Co. (Japan)	natural origin fibers such as cotton with antibacterial and antifungal activity
Amicor AB, Amocor AF, Amicor Plus / Acords (England)	polyacrylic fibers with antibacterial and antifungal activity
Fibra K/ Asach Chemical Ind.Co (Japan)	viscose silk with colloidal sulfur with antibacterial activity
Gymlene/F.Drake Fibres (England), Microban/ Filament Fiber (USA)	polypropylene with antibacterial activity
Rhodia/ Rhodia Technical Fibres (Germany), Livefresh/ Kanebo (Japan)	polyamide with antibacterial activity
Huvis Corp. (Korea), Bacterkiller/ Kanebo (Japan), Kuraray/ Kuraray (Japan) , Trevira Bioactive / Trevira (Germany)	polyester with antibacterial activity
Rhovyl' AS/ Rhovyl (France)	PVC with antibacterial activity

Table 4. Nonwovens with antimicrobial activity (available on the global market)

Nonvowens	Antimicrobial agents	Antimicrobial activity	Author, year
Cotton	phosphonium salts with alkyl chains; chloroacetyl chloride, naphtylacetic acid, alginate-quaternary ammonium complex	active against *Staphylococcus aureus, Escherichia coli*	Jantas & Górna, 2006;Kanazawa et al., 1994; Kim et al., 2010
Polyurethane, Polyglicidyl methacrylate	quaternary ammonium salts with aliphatic triisocyanate, phosphonium salts	active against *S.aureus, E.coli, Pseudomonas aeruginosa, Bacillus subtilis, B.cereus, Shigella* sp., *Salmonella typhi,*	Kenawy et al., 2002; Kenawy & Mahmoud, 2003; Nurdin et al., 1993

Nonvowens	Antimicrobial agents	Antimicrobial activity	Author, year
		Trichophyton rubrum, Candida albicans, Aspergillus flavus, Fusarium oxysporium	
Polypropylene, Polypropylene-cotton	glycidal methacrylate, β-cyclodextrin, quaternary ammonium – chitosan complex, chitosan,	polypropylene with glycidal methacrylate, β-cyclodextrin, quaternary ammonium –chitosan complex active against *Lactobacillus plantarum, S.aureus, E.coli;* polypropylene with chitosan active against *S.aureus, E.coli, Proteus vulgaris,* not effective against: *Klebsiella pneumoniae, P.aeruginos;.* polypropylene-cotton with chitosan active against *Fusarium oxysporum, Verticillium alboatrum, Alternaria alternata, Clavibacter michiganensis, Pseudomonas solantacearum*	Abdou et al., 2005; Kim et al., 2010
Cotton	chitosan	active against *S.aureus*	Lim & Hudson, 2004
Cotton	silver, nanosilver	active against *Candida albicans*, *C.tropicalis, S.aureus, E.coli, K.pneumoniae, Streptococcus faecalis*	Gorensek & Recelj, 2007; Hipler et al., 2006; Sachinvala et al., 2007
Wool	silver and nanotitanium dioxide photo-induced	active against *S.aureus, E.coli,*	Montazer et al., 2011
Nylon, silk	nanosilver	active against *S.aureus*	Dubas et al.,2006
Poliacrylonitryle, poli(N-vinyl-pyrrolidone), PVC, cellulose acetate, Poliester, Polycaprolactone Polyurethane, Polipropylene	nanosilver, lidocaine, gold, zinc oxide nanotitanium dioxide	active against *S.aureus, E.coli, P.eruginosa*	Jain & Pradeep, 2005; Lala et al., 2007; Radetic et al., 2008, Yu et al., 2003
Phosphate glass fiber	copper (CuO)	active against *Staphylococcus epidermidis*	Abdou-Neel et al., 2005

Nonvowens	Antimicrobial agents	Antimicrobial activity	Author, year
Polypropylene , polypropylene with cotton	4-vinyl pyridine, radiation-induced	active against *E.coli,* depended on the structure and content of pyridinium groups, not bactericidal, but bacteriostatic	Tan et al., 2000
Cotton	N-halamine	active against *S.aureus, E.coli,*	Ren et al., 2009
Poly(L,L-lactide) on viscose	triclosan	active against *S.aureus, E.coli*	Goetzendorf-Grabowska et al., 2004
Polypropylene, polyacrylonitryle	tetracycline hydrochloride, vinyloimidazol, ciprofloxacin	active against *S.aureus, E.coli, K.pneumoniae*	Gupta et al., 2007, 2008
Poly(ethylene terephtalate)	cephalosporin	active against *S.aureus, E.coli, P.aeruginosa*	Bucheńska et al., 2003
Polyamide, polypropylene, polyester	clotrimazol, ketokonazol	active against *C.albicans, Penicillium funiculosum, P.mycetomagenum, Aspergillus niger, A.repens T.mentagrophytes*	Struszczyk et al., 2003

Table 5. Nonwovens with antimicrobial agents (based on the scientific researches)

Nonvowens	Antimicrobial agents	Antimicrobial activity	Author, year
Silk	Dyes from plants *Morinda citrifolia; Terminalia catappa, Artrocarpus heterophyllus, Tectona grandis* (contain of: flavonoids, quinonoids, indigoids, tannins)	active against *E.coli, K.pneumoniae, C.albicans, A.niger*	Prusty et al., 2010
Wool	Dyes Catechu from *Acacia catechu* (main component catechin)	active against *E.coli, S.aureus, C.albicans, C.tropicalis*	Khan et al., 2011
Wool	Dyes from *Acacia catechu, Kerria lacca, Quercus infectoria, Rubia cordifolia, Rumex maritimus*	active against *E.coli, B.subtilis, K.pneumoniae, Proteus vulgaris, P.aeruginosa*	Singh et al., 2005
Wool	Dye curcumin from *Curcuma longa*	active against *E.coli, S.aureus,*	Han & Yang, 2005
Cotton	Dyes from *Acacia catechu, Kerria lacca, Mallotus philippinensis, Punica granatum, Quercus infectoria, Terminalia chebula, Rheum emodi*	active against *E.coli, K.pneumoniae, Proteus vulgaris,*	Gupta et al., 2004

Nonvowens	Antimicrobial agents	Antimicrobial activity	Author, year
Chitosan and viscose	Flavonoids (flavanols, flavonol, flavone, flavanone, isoflavanone)	active against *B.subtilis, P.aeruginosa*	Sousa et al., 2009
Wool, cotton	Dye: Citrus grandis Osbeck extract	active against *S.aureus, K.pneumoniae,*	Yi et al., 2010
Cotton	Neem seed extract from *Azadirachta indica* (contain of:: azadirachtin, nimbin,, nimbidin, salannin, nimbidol, gedunin)	active against *B.subtilis, P.vulgaris,*	Joshi et al., 2007

Table 6. Nonwovens with natural origin antimicrobial agents (based on the scientific researches)

6. Factors affecting on the activity of biocides in the fibers

The activity of biocides depends on many factors, of which the most important include time of contact with the microorganisms, concentration of active substance, type of microorganism, presence of organic and inorganic impurities, temperature, humidity and pH.

The most important factors for affecting biocidal activity are **time** of contact between the active substance and the microorganism cells, and the biocide **concentration** (Brycki, 2003).

The product of the concentration and time of action for specified groups of active substances is a constant value, expressed in terms of Watson's equation:

$$c^{\eta} \times t = \text{const.} \quad (1)$$

where c denotes concentration, t denotes time, and ŋ is a concentration coefficient determined empirically for a given substance.

Example values of the concentration coefficient (ŋ) are 10 for alcohols, 6 for phenols, and 1 for quaternary ammonium salts.

The use of this relationship is important from a practical standpoint – it tells us that given an appropriate concentration of biocide, a biocidal effect will be achieved in a precisely specified time. With a preparation based on alcohol, for example, if it were diluted to half of the concentration, the length of time required to obtain the same effect would increase by 1024 times. In the case of phenol it would increase by 64 times, and for quaternary ammonium salts it would merely double. Because of their properties, alcohols work effectively for a short time, and hence their use is limited to short-lasting disinfection (Brycki, 2003).

With regard to the uses of bioactive fabrics, either a short time of action on microorganisms is required (for example, in the case of protective masks the time should not exceed 8 hours), or the time may be extended to 24–48 hours (filtration and technical materials, etc.).

The effective action of a biocide also depends on the **type of microorganisms**, chiefly the structure of the cell wall and the presence of genetic resistance mechanisms. For this reason, research into the antimicrobial activity of fabrics should include evaluation with respect to different species of microorganisms (Table 7).

Microorganisms	N_t After 6 h of incubation	
	Mean	**SD**
Escherichia coli	0.000	0.000
Pseudomonas aeruginosa	0.001	0.000
Klebsiella pneumoniae	0.001	0.000
Staphylococcus aureus	0.000	0.000
Micrococcus flavus	0.000	0.000
Bacillus subtilis	**0.550**	**0.348**
Candida albicans	0.000	0.000
Aspergillus niger	**0.036**	**0.006**
Penicillium chrysogenum	**0.004**	**0.001**

SD – standard deviation
*needle-punched nonwoven; polypropylene-silver (in the form of master batches) + acrylic fiber-biocide

N_t: $N_t = \frac{N}{N_0}$ where N_0 — the number of microorganisms on the sample of the textile material for time $t = 0$, N – the number of microorganisms on the sample of the textile material for time t_n

Table 7. Microorganisms Survival Index (N_t) for various microorganisms after 6 hours incubation with bioactive nonwoven* (based on Majchrzycka et al., 2010)

Microorganisms sensitive to the action of biocides are bacteria – Gram-positive cocci and Gram-negative bacilli. The most resistant organisms, with high survival rates, include spore-forming bacteria and moulds (Majchrzycka et al., 2010). This is because the activity of biocides added to fabrics is dependent on the physiological state of the microorganisms: the most sensitive are cells in a phase of vegetative growth, while resistance is shown by endospore of bacteria and the spores of moulds (Gutarowska et al.,2010).

Organic contaminants present on the fabric may reduce the biological effect. Proteins are substances that protect microorganisms, sugars and fats may be a source of food and lead to the development of microorganisms, and moreover those compounds may react with the biocides, reducing their effectiveness. Research into antimicrobial activity in the presence of artificial sweat (inorganic compounds) did not reveal any significant effect on the bioactivity of fabrics (Majchrzycka et al., 2010).

Increased **temperature** generally strengthens the antimicrobial activity of chemical agents, due to the increased reactivity of the active substances as well as synergy between the destructive effects of the substance and temperature (Brycki, 2003).

Increased **humidity** strengthens the antimicrobial activity of fabrics containing biologically active substances. The presence of water makes it possible for the biocide to penetrate into the cells of microorganisms in the form of ions, and for these to act effectively. Hence fibres with **hydrophilic** properties containing biocides will be more effective than hydrophobic fibres containing the same active substances (Gutarowska et al., 2010). Comparative studies on the antimicrobial action of hydrophobic PAN fabrics containing quaternary ammonium salts and the same fabrics containing biocide on an inorganic medium – perlite – with hydrophilic properties showed a significant improvement in the biocidal effectiveness of hydrophilic fabrics with added perlite (Table 8). Bioactivity improved with increasing concentration of perlite, which changed the properties of the fabric to hydrophilic, and with increasing humidity of the fabric (Table 9).

Amount of bioperlite in the nonwoven (%)	**Amount of alkylammonium microbiocides in the nonwoven (%)**	**Number of bacteria (CFU/sample)**					
		Escherichia coli			***Staphylococcus aureus***		
		Incubation time		Reduction %	Incubation time		Reduction %
		0 h	6 h		0 h	6 h	
Control without bioperlite	0	Mean: 6.07×10^6 SD: 3.52×10^6	Mean: 1.59×10^6 SD: 1.53×10^5	73.80	Mean: 3.33×10^6 SD: 2.91×10^6	Mean: 1.13×10^6 SD: 1.02×10^6	66.0%
Nonwoven bioperlite 5 %	0.23	Mean: 3.72×10^6 SD: 2.35×10^6	Mean: 2.76×10^5 SD: 1.49×10^5	95.45	Mean: 5.01×10^6 SD: 3.35×10^6	Mean: 2.34×10^5 SD: 2.05×10^5	92.97
Nonwoven bioperlite 10 %	0.46	Mean: 7.23×10^5 SD: 1.13×10^5	Mean: 0 SD: 0	100	Mean: 5.29×10^6 SD: 4.58×10^6	Mean: 0 SD: 0	100
Nonwoven bioperlite 15 %	0.69	Mean: 8.67×10^5 SD: 1.05×10^5	Mean: 0 SD: 0	100	Mean: 1.84×10^7 SD: 3.12×10^6	Mean: 0 SD: 0	100
Nonwoven bioperlite 20 %	0.93	Mean: 7.05×10^5 SD: 1.20×10^5	Mean: 0 SD: 0	100	Mean: 2.43×10^6 SD: 1.56×10^6	Mean: 0 SD: 0	100

SD – standard deviation

Table 8. The influence of bioperlite concentration (with alkylammonium microbiocides) in the nonwoven on antimicrobial activity against *E.coli* and *S.aureus* (based on Gutarowska et al., 2010)

Nonwoven mass humidity level (%)	**Number of microorganisms (CFU/sample)**		**Reduction (%)**
	Incubation time		
	0 h	6 h	
Nonwoven (5%) control*	Mean: 2.04×10^4 SD: 1.80×10^4	Mean: 4.15×10^3 SD: 5.60×10^3	79.66
Nonwoven (9.5%)	Mean: 3.52×10^4 SD: 3.08×10^4	Mean: 6.96×10^3 SD: 6.03×10^3	80.23
Nonwoven (43%)	Mean: 2.04×10^4 SD: 1.78×10^4	Mean: 3.54×10^3 SD: 3.50×10^3	82.64
Nonwoven (213%)	Mean: 2.04×10^4 SD: 1.80×10^4	Mean: 3.44×10^3 SD: 3.68×10^3	83.14
Nonwoven (1274%)	Mean: 2.04×10^4 SD: 1.80×10^4	Mean: 1.87×10^2 SD: 1.71×10^2	99.08

*without the addition of water; SD – standard deviation

Table 9. The influence of the humidity level of a nonwoven with 8% bioperlite on antimicrobial activity against *E.coli* (Gutarowska et al., 2010)

The impact of **pH** on biocidal activity depends on the chemical nature of the compound; it may have both positive and negative effects. In the case of phenol compounds an increase in pH causes a reduction in antimicrobial activity, although such a change causes an increase in the activity of quaternary ammonium salts (Brycki, 2003).

Of significant importance for the biological activity of fabrics is the way in which the biocide is introduced into the fabric. The **carriers** for active substances are highly significant. Sample tests with the use of several mineral carriers for silver have shown significant differences in the antimicrobial activity of the resulting fabrics (Gutarowska & Michalski, 2009). In these studies, it was observed the best biocidal effects against test microorganisms (*E.coli, S.aureus, C.albicans, A.niger*) characterized the nonwovens containing silver on TiO2 and BaSO4 carriers (BT nonwovens) and nonwovens with silver on TiO2 and ZnO carriers (TL nonwovens) (Fig.2).

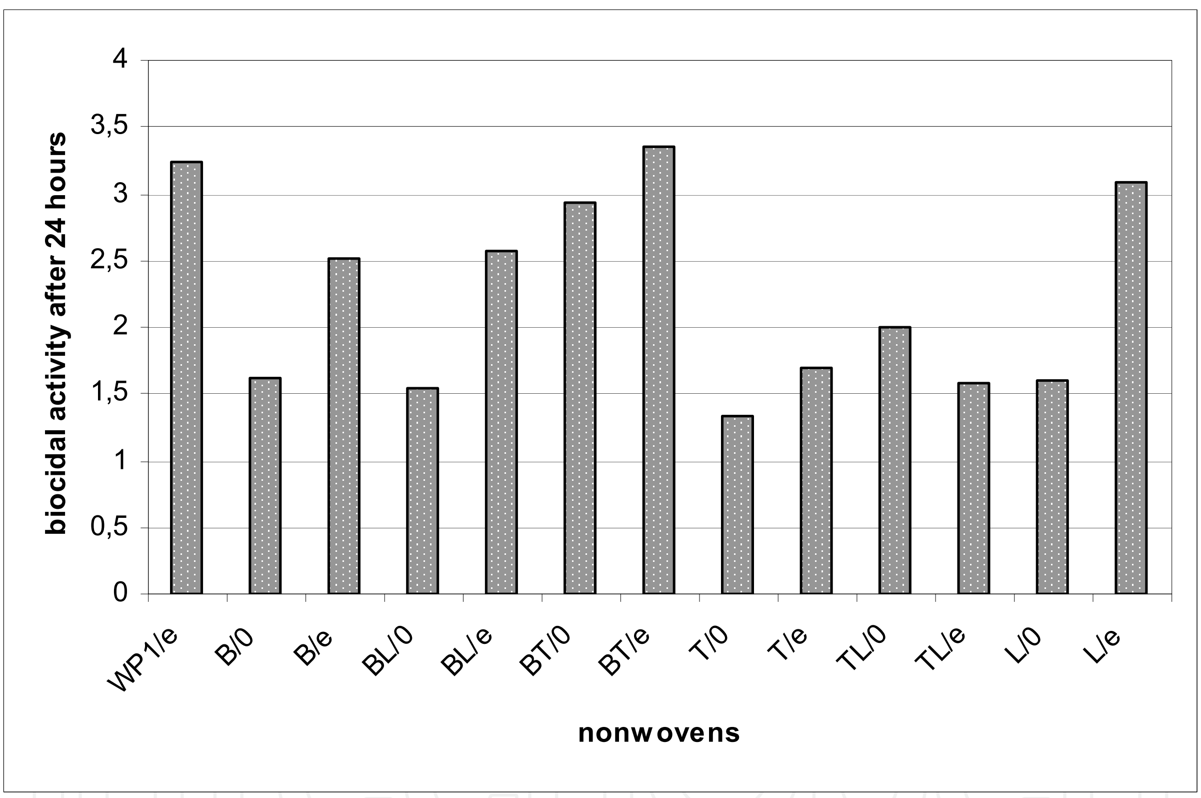

Fig. 2. Biocidal activity of nonwoven with biocides (Ag), to bacteria E.coli after 24 hours incubaction with nonwoven (Gutarowska & Michalski, 2009)

Legend:

Sample code	Added concentrate containing 30% Ag/AgCl with a carrier/ Presence of static charge (no: -; yes:+)
WP1/0	Control without concentrate, (-)
WP1/e	Control without concentrate, (+)
B/0	$BaSO_4$ (-)
B/e	$BaSO_4$ (+)
T/0	TiO_2 (-)

T/e	TiO_2 (+)
L/0	ZnO (-)
L/e	ZnO (+)
BT/0	$BaSO_4$ + TiO_2 (-)
BT/e	$BaSO_4$ + TiO_2 (+)
BL/0	$BaSO_4$ + ZnO (-)
BL/e	$BaSO_4$ + ZnO (+)
TL/0	TiO_2 + ZnO (-)
TL/e	TiO_2 + ZnO (+)

Generally it was observed that appropriate selection of two carriers improves the effectiveness in comparison with nonwovens in which a single carrier was used. Good effect was reflected both by high biocidal activity and by reduced time off effective contact of microorganisms with the nonwoven. High activity was obtained for the majority of nonwovens with electrostatic charge against bacteria (BL/e, BT/e, T/e, L/e) and for all nonwovens with charge against fungi.

Active substances can be added to fabrics in different ways:

1. Physical modification – introduction of an active compound into the spinning solution or molten fibre-forming polymer and closure within the fibre (occlusion). The biocidal substance then diffuses to its surface, where it acts on the microorganisms.
2. Chemical modification – chemical reactions on the finished textile product, bonding of the biocide through the formation of chemical bonds, e.g. introduction of metal particles to zeolites added during fibre formation, addition of antibiotics to modified fibres by way of grafted copolymerization.
3. Finishing – application of a poorly soluble coating, with the use of a polymeric or low-molecular-weight medium with which the biocide is bonded physically or chemically.
4. Microencapsulation – the introduction into textiles of microcapsules containing volatile substances, dyes with antimicrobial action (Nelson, 2002).

In the case of the first method the biocides must be chosen to have suitable properties so that the technological process (high temperature) does not cause inactivation of the compound: many chemical substances display volatility at high temperatures. This method gives a long-lasting biological effect, as the biocides are permanently fastened to the fibre matrix. Chemical modification of a polymer by acetylation/phosphorylation makes it possible to obtain fibres with permanent antimicrobial properties. However due to the high costs of the production technology, and frequent change in the strength parameters of fabrics, these methods are rarely used. The most popular method is the application of a biocidal finishing layer. The use of a finish on the surface of the finished product favours high antimicrobial activity, although such a product does not retain its properties for a long time, losing them during successive washing cycles (Szostak-Kot, 2004).

The choice of method of producing a fabric should depend on its intended use. Textiles meant for repeated use (socks, bed linen, aprons, underwear, towels) should be highly wash-resistant; in these case the biocides must be permanently joined to the fibre matrix, in contrast to disposable items (aprons, masks, filters, bandages, dressings, gauzes and hospital foot coverings).

7. Methods for evaluating anti-microbiological activity of nonwovens

The need to produce bioactive textiles containing biocides has led to the development of methods for evaluating antimicrobial activity. The final result of such a test is highly dependent on the testing method and the choice of test microorganism. Methods of evaluating antimicrobial properties can be divided into quantitative and qualitative methods (Dymel et al., 2008; Gutarowska et al., 2009).

Evaluation of the antimicrobial activity of textile products by qualitative methods is based on observation of the growth of microorganisms under and around a sample placed on an agar medium with a culture of the microorganisms. The effect of antimicrobial activity is indicated by the variously sized area in which the growth of the microorganisms is suppressed (Photographs 1–3).

Qualitative methods make it possible to evaluate the biocidal action of textiles both in the form of flat products, namely unwoven, woven and knitted fabrics, and in the form of fibres, threads, etc. The hydrophilic or hydrophobic nature of the textiles also has no effect on the final result. The only criterion for a textile product to be tested by qualitative methods is the diffusion of the active substance into the medium. Products must demonstrate at least minimal diffusion of the active component.

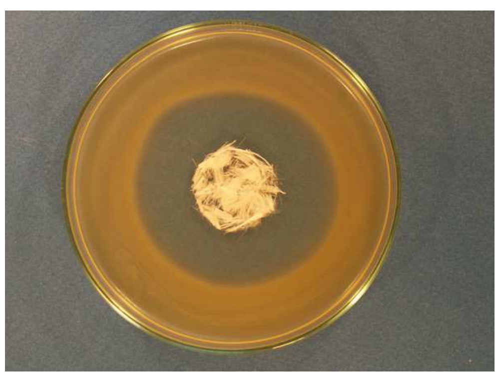

Photo 1. Growth inhibition zone around the *S. aureus;* polypropylene fibers containing 2% Ingaguard- method according to SN 195 920

Photo 2. Growth inhibition zone around the *C. albicans* polypropylene fibers containing 2% Ingaguard- method according to SN 195921

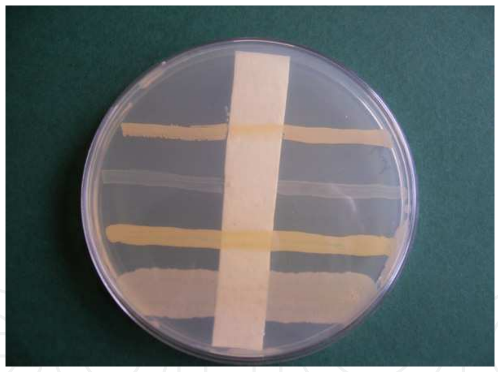

Photo 3. Growth of bacteria respectively from the top: *S. aureus, E. coli; M.flavus, B. licheniformis* under polymer with nano-silver - method according to AATCC 147

Table 10 lists the most commonly used qualitative methods, including Swiss (SN), American (AATCC), Japanese (JI) and European (EN ISO) methods.

Method / standards	**Standard number**
Antifungal activity, assessment of textile materials: Mildew and rot resistance of textile materials	AATCC 30
Antibacterial activity of fabrics, detection of: Agar plate method	AATCC 90
Antimicrobial activity assessment of textile materials: Parallel streak method	AATCC 147
Antimicrobial activity assessment of carpets	AATCC 174
Standard Test Method for Using Seeded-Agar for the Screening Assessment of Antimicrobial Activity In Carpets	ASTM E2471-05
Standard Test Method for the Assessment of Antimicrobial Activity In Carpets; Seeded-Agar Overlay Screen	ASTM WK4757
Resistance of Textiles to Microbiological Attack. Textiles – Determination of the antibacterial activity – Agar plate diffusion test	CEN/TC 248/WG13
Testing for antibacterial activity and efficacy on textile products	JIS L 1902
Textile fabrics: Determination of the antibacterial activity: Agar diffusion plate test	SN 195920
Textile fabrics: Determination of the Antimycotic Activity: Agar Diffusion Plate Test	SN 195921

Table 10. Qualitative methods for assessing antimicrobial activity of bioactive nonwovens

Qualitative testing methods are similar to each other. They involve pouring out a layer of agar inoculated with a bacteria culture or fungal spores of specified density, or the application of microorganisms on an agar plate via linear inoculation. The tested material and a control sample of specified size are then placed on the inoculated medium. Following incubation, the action of the biocide is evaluated by measuring the area of suppression of growth, compared with a control sample not containing active antibacterial agent.

Quantitative methods are based on the general principle of inoculating the tested sample of material with a suspension of microorganisms of specified density, and then incubating them with the fabric. After some time, based on the number of microorganisms which survived contact with the fabric, the activity of the biocide in the sample is determined relative to a control sample not containing biocide. Quantitative methods are superior to qualitative ones, as the numerical results obtained for the biological activity of unwoven fabrics and textiles can be compared, to select the most effective solution for eliminating microorganisms. Table 11 lists the quantitative methods used for determining the antimicrobial activity of bioactive unwoven fibres and textile products.

Method / standards	Standard number
Assessement of antibacterial finishes on textile materials	AATCC 100
Testing for antibacterial activity and efficacy on textile products	JIS L 1902
Testing hygienically-treated textile products for effectiveness against bacteria. Textile products hygienic finish council	Shake Flask Method
Properties of textiles-Textiles and polymeric surfaces having antibacterial properties. Characterization and measurement of antibacterial activity	XP G39-010
Textile fabrics: Determination of the antibacterial activity: Germ count method	SN 195924
Testing for antibacterial activity	ISO/TC 38/-/WG23
Antimicrobial products – Test for antimicrobial activity and efficacy	JIS Z 2801:2000

Table 11. Methods for quantitative assessment of antimicrobial activity of bioactive nonwovens and textiles

The choice of method cannot be a random one; it is chiefly dependent on such criteria as the type of fabric, its properties and intended use, and the time of action on microorganisms. Based on these criteria and on analysis of the quantitative methods for evaluating antimicrobial properties of bioactive fabrics, a decision chart has been drawn up to enable the selection of an appropriate testing method (Figure 3).

The test results Method AATCC 100 and Shake Flask Method are stated relative to the surface area or mass of the sample, in terms of reduction in the quantity of microorganisms:

$$\%\ \text{reduction} = (N_0\text{-}N)/N_0 \times 100\% \quad (2)$$

where:

N_0 is the number of microorganisms per sample at time t_0 with the bioactive fabric, and N is the number of microorganisms per sample after a time t_n of exposure with the bioactive fabric.

A positive evaluation is given to fabrics on which the reduction in microorganisms is greater than 85%.

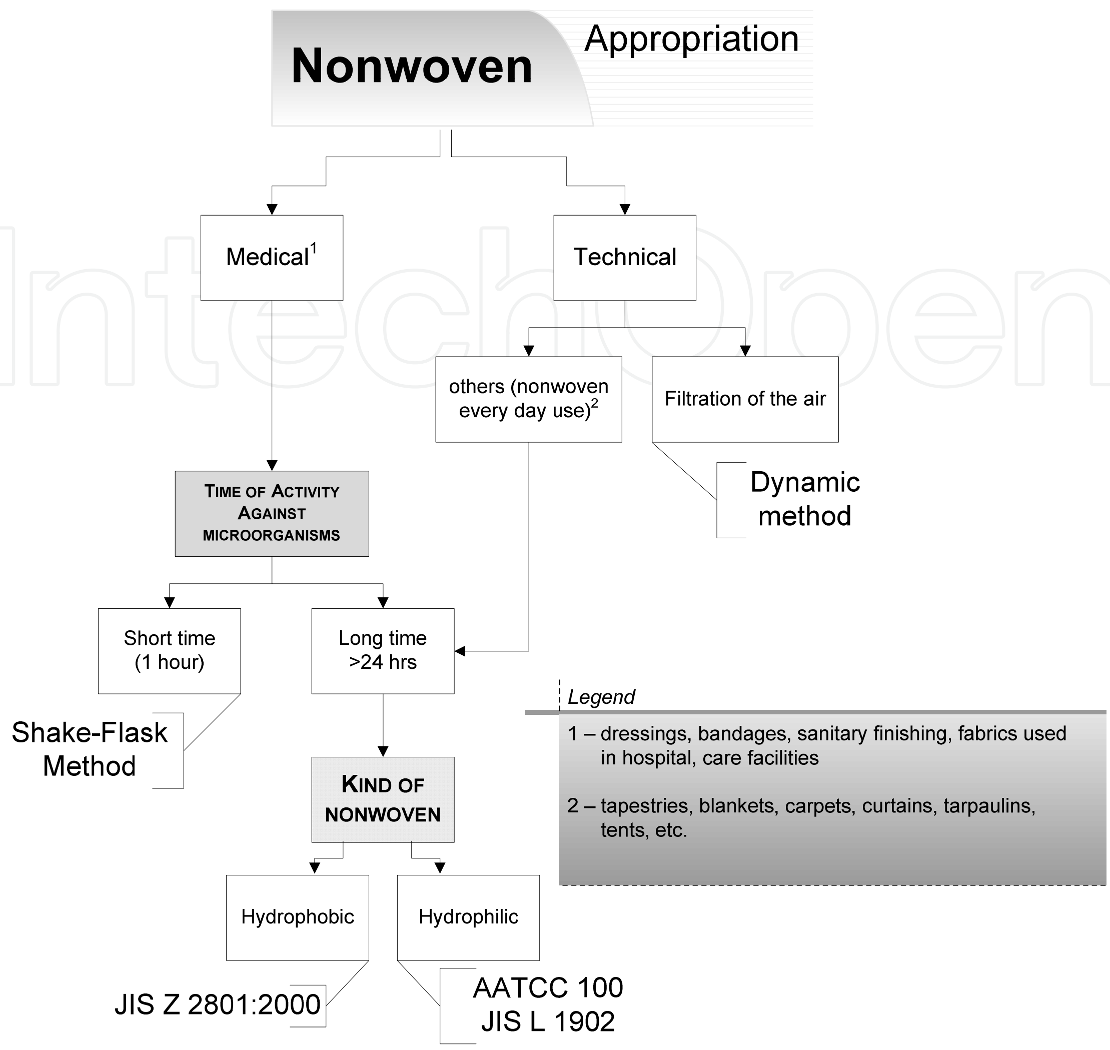

Fig. 3. A decision tree for choose the method of quantitative evaluation of antimicrobial activity of bioactive nonwoven (based on Gutarowska et al. 2009)

The result may be given in the form of bactericidal activity and bacteriostatic activity (Method JIS L 1902, JIS Z 2801:2000).

Biostatic activity is calculated from the formula:

$$\text{biostatic activity (S)} = \log N_k/N \quad (3)$$

where:

N_k is the number of microorganisms per sample after a time t_n of exposure with the control fabric, and N is the number of microorganisms per sample after a time t_n of exposure with the bioactive fabric.

Biocidal activity is calculated analogously:

$$\text{biocidal activity (L)} = \log N_0/N \quad (4)$$

where:

Microorganism	Pathogenicity	Characteristic
Escherichia coli ATCC 11229	digestive disorders, urinary tract infections	reference strain, gram negative rods, a significant resistance to biocides
Pseudomonas aeruginosa	pathogen, various types of infection, inflammation of the skin and nosocomial infections	reference strain, gram negative rods, a significant resistance to biocides
Klebsiella pneumoniae	pathogen, pneumonia, transmitted by air, nosocomial infections	reference strain, gram negative rods
Staphylococcus aureus ATCC 6538	pathogen, dermatitis, pneumonia, venous blood clots, ulcers, myocarditis, transmitted by air, common carriers in the nasal cavity and throat, nosocomial infections	reference strain, gram positive coccus
Staphylococcus epidermidis	saprophyte, harmless to health, sometimes skin infections	gram positive coccus, exists on the skin
Micrococcus flavus	saprophyte, harmless to health	gram positive coccus, often isolated from the air, high resistance to UV and disinfectants
Bacillus subtilis	saprophyte, harmless to health, sometimes causes digestive disorders	gram positive bacilli produces spores, often found in the environment (air, soil),
Candida albicans	a potential pathogen, systemic infections, skin, nail mucous membranes infections, hypoallergenic	reference strain, yeast, widespread in the environment (mucous membranes, air, skin)
Rhodotorula rubra	saprophyte, harmless to health, sometimes skin infections	yeast, widespread in the environment (air, food)
Aspergillus niger	saprophyte, harmless to health, sometimes respiratory, cornea and skin infections	mould, reference strain for testing of technical material resistance, present in the air
Penicillium chrysogenum	saprophyte, harmless to health, sometimes upper respiratory tract infections, ear and nail infections, allergies	mould, often isolated from air
Alternaria alternata	saprophyte, harmless to health, hypoallergenic	mould, often isolated from air
Trichophyton mentagrophytes	pathogen, infections of hair, skin and nails	mould, reference strain
Scopulariopsis brevicaulis	pathogen, nail, skin and mucous membranes infections	mould
Epidermophyton floccosum	pathogen, infections of hair, skin and nails	mould

Table 12. Characteristics of test microorganisms for determination of the antimicrobial activity of bioactive nonwovens (based on: Gutarowska et al., 2009)

N_0 is the number of microorganisms per sample at time t_0 with the bioactive fabric, and N is the number of microorganisms per sample after a time t_n of exposure with the bioactive fabric.

A sample is taken to have bactericidal properties if the value of the coefficient of bactericidal activity (L) is greater than zero, and to have bacteriostatic properties if the value of the coefficient of bacteriostatic activity (S) is greater than 2 (Yu, 2003), which denotes a 100 fold reduction in the number of microorganisms.

The evaluation of activity is made with respect to selected potentially pathogenic (from Pure Culture Collections ATCC, NCTC) or saprophytic microorganisms occurring naturally in the human environment. Table 12 lists test microorganisms used for evaluation of the bioactivity of textiles and for their description.

The fundamental criterion for the selection of microorganisms for testing of antimicrobial activity is the intended use of the fabric. In the case of therapeutic fabrics, coming into contact with the human skin, or intended for use in hospitals and care centres, the microorganisms chosen for testing are those which are pathogenic and which are particularly resistant to chemical disinfection and antibiotic treatment, leading to hospital infections, for examples: *Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli, Bacillus licheniformis, Corynebacterium xersosis, Trichophyton mentagrophytes, Candida albicans*. Technical fabrics for uses such as air filtration, and for everyday uses (upholstery, blankets, carpets, net curtains, tarpaulin, etc.) usually come into contact with saprophytic microorganisms, not hazardous to human health, which are constantly present in the air in the form of bioaerosols. Such fabric is tested against the fungi: *Aspergillus niger, Penicillium chrysogenum, Alternaria alternata, Cladosporium cladosporioides* and bacteria*: Micrococcus flavus, Bacillus subtilis*.

8. Conclusions

Biodeterioration of textile materials, mainly natural origin is a serious global economic problem. It requires long-term protection of these materials against destructive activity of microorganisms. At the same time the high standards of hygiene in some areas, primarily medicine, at the work places and others, requires the use of textile materials with antimicrobial properties. In recent years the number of studies on the new biocides and technology of textiles production with antimicrobial activity has increased. The requirements for modern fabrics with antimicrobial properties include high efficiency. In this area the effective methods for proper localization of chemical preparations have been developed, eg microencapsulation or by increase of the surface of preparation, eg by using the active agent in the form of nanoparticles. Most research has been focused on the searching for the new agent - biocides with high efficiency, which are not only effective but also safe, which don't cause the skin irritation, respiratory allergy. Future application will be concentrated on the natural origin substances. The attention also should be done on the biodegradability and environmental protection.

9. References

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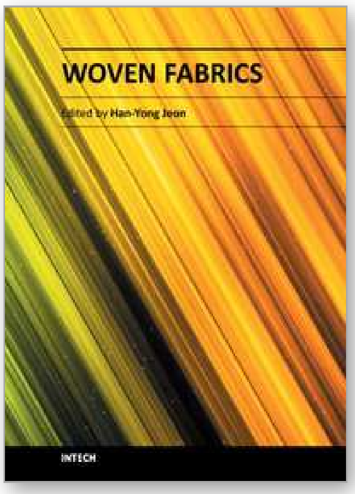

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"Woven Fabrics" is a unique book which covers topics from traditional to advanced fabrics widely used in IT, NT, BT, ET, ST industry fields. In general, woven fabrics are known as the traditional textile fabrics for apparel manufacturing and are used widely in various fabric compositions as intermediate goods that affect human activities. The relative importance of woven fabrics as traditional textile materials is extremely large and currently application fields of woven fabrics as technical textiles are rapidly expanded by utilizing its geometric features and advantages. For example, the book covers analytical approaches to fabric design, micro and nano technology needed to make woven fabrics, as well as the concept for industrial application.