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Antimicrobial Properties of Socks Protected with Silver Nanoparticles

Abstract
Antimicrobial properties of socks containing silver nanoparticles were investigated. Two types of socks were used for testing. The first were linen (100%) socks impregnated with a specimen containing silver nanoparticles. The second type were commercially available cotton (55%) socks containing nanosilver. An antimicrobial effect was assayed against selected Gram-positive and Gram-negative bacteria as well as yeasts. It was found that the specimen used for impregnating linen socks has a wide range of antimicrobial activity against some Gram-positive, Gram-negative bacteria and yeasts - Candida albicans. Antimicrobial effectiveness depended on the type of microorganism, cell number and concentration of silver nanoparticles. Commercially available cotton socks presented antibacterial properties against Staphylococcus epidermidis.

Key words: *silver nanoparticles, antibacterial, cotton socks, linen socks, textiles.*

Introduction

In the past few decades, researchers have taken an interest in the development of textile fabrics containing antibacterial agents [1]. Different types of substances are used in the textile industry, for example oxidising agents, coagulants, and metallic or quaternary ammonium compounds [2]. Many of them are possibly harmful or toxic, while nanosilver is considered as relatively safe [3].

Silver nanoparticles have some unique chemical and physical properties such as a high surface area, small size (< 20 nm) and high dispersion, which make them an effective antimicrobial agent [4 - 7]. Moreover, it is believed that silver has a wide spectrum of activity including bacteria, fungi and viruses [8]. Silver nanoparticles can be used in different forms, for example as a colloid solution or they can be incorporated into some materials [9]. That is why they are commonly used in a variety of products from medical devices to clothes and textiles [3, 10].

The exact mechanism of antimicrobial activity is not fully explained and is an expanding field of research [8]. It is suggested that the mechanism of antibacterial effects involves the absorption and accumulation of silver nanoparticles by cells causing structural changes in the cell membrane and dissipation of the proton motive force. They also interact with the S-H bounds, blocking and denaturing proteins [11].

Textile materials made from natural fibres such as cotton or linen are a good environment for the growth of microorganisms, including pathogenic bacteria and fungi, especially in contact with human skin, which provides them with moisture, warmth and nutrients [12]. Therefore the incorporation of silver nanoparticles into textiles has received great interest.

In some studies it was reported that cotton fabrics containing silver nanoparticles exhibited antibacterial activity against *Staphylococcus aureus* [13], *Escherichia coli* [14], *Bacillus subtilis* and *Pseudomonas putida* [15], or against fungi *Aspergillus niger* [15]. Lee et al. [16] investigated the antibacterial effect of nanosized silver on cellulosic and synthetic textile fabrics against *S. aureus* and *Klebsiella pneumoniae.* Antibacterial properties of textiles were achieved by using a padding process [16] or through the use of a sol-gel process [14, 15]. However, in some studies it was found that the values of bacterial growth inhibition are reduced during washing cycles. This problem was overcome by incorporating a binder into the finishing formulation [17, 18]. There are different studies on the properties of fibres and fabrics containing silver nanoparticles [19 - 22], indicating their possible applications.

The application of silver nanoparticles in commercial products has been increasing [23]. At the same time, there is increasing concern about exposure to engineered nanoparticles and their effect on humans and the environment [24 - 27]. One of the most important issues is the release of silver nanoparticles from commercial fabrics [28, 29]. Benn and Westerhoff investigated silver released from socks into water, and its fate in wastewater treatment plants [30]. In another study [30] antimicrobial activity of a AgPURE™ specimen was verified for the purpose of preventing microbial odours. AgPURE-Nanosilver is a proven auxiliary for the biologically active finishing of textiles according to Oeko-Tex® Standard 100 [30, 31].

The aim of the work presented was to investigate the antimicrobial properties of linen socks impregnated with a silver nanoparticle specimen and compare with those of commercial cotton socks containing nanosilver.

Materials and methods

Tested materials

Two types of socks were used in experiments:

- linen socks (100% linen, producer: Harter Natur Textilien, Germany), (without nanosilver)
- Silver SeaCell socks (producer: JJW s.j., Poland). The product contains: 55% cotton, 23% Lyocell with algae, 19% polyamide, 3% polyurethane, silver (4 ppm).

A NANOfresh specimen (producer: NANOBIZ.PL) was used for the impregnation of the linen socks. The producer describes this product as an odour-fighting specimen with silver nanoparticles to be used on clothes and fabrics. The product contains < 2% vegetable complexing agents and silver nanoparticles NPS100 (5 ppm), i.e. the nanoparticle size was below 100 nm.

Table 1. *Antimicrobial effect of the NANOfresh specimen on the growth of indicator microorganisms.*

Test species	Diameter of zone inhibition determined by well diffusion assay, mm	Minimal inhibitory concentration of NANOfresh specimen, ppm	
		Cell density 10^5 CFU ml^{-1}	Cell density 10^8 CFU ml^{-1}
Staphylococcus aureus	3.9 ± 0.6	0.5	
Staphylococcus epidermidis	6.7 ± 0.6	0.125	
Bacillus megaterium	3.8 ± 0.5	> 0.5	
Pseudomonas aeruginosa	3.6 ± 0.6	0.25	
Candida albicans	2.5 ± 0.4	> 0.5	

Microorganisms and media

The experiments were performed on five microorganisms characteristic for human skin or commonly present in the environment (air, dust): Gram-positive bacteria *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus megaterium,* Gram-negative bacteria *Pseudomonas aeruginosa*, and yeasts - *Candida albicans*. Nutrient agar or broth was used for bacterial cultivation and wort agar for yeast culture.

Before the experiments, all microrganisms were subcultured on fresh media suitable for a given microorganism and then incubated for 24 hours in a temperature of 37 °C (bacteria) or 30 °C (yeasts). Next suspensions of microorganisms in saline were prepared and their density was established at a level depending on the experiment.

Antimicrobial activity assay of NANOfresh specimen

Antimicrobial activity of the NANOfresh preparation was evaluated by well diffusion assay. Suspensions of microorganisms were overlaid with agar media and 1 cm diameter wells were cut in the agar with a cork-borer. 100 µl of the specimen tested was introduced into each well and then all plates were incubated in appropriate conditions. After 24 hours in the case of bacteria and 48 hours in the case of yeasts, zones of inhibition were measured. The experiment was repeated three times, mean values of which are presented.

Evaluation of the relationship between the concentration of the NANOfresh specimen and cell number of bacteria and yeast

The effect of test preparation on the growth of the microorganisms examined depending on the amount of nanosilver and cell number was evaluated by the method of serial dilutions on microtitre plates. First, serial two-fold dilutions of the NANOfresh specimen were made in sterile water, obtaining solutions with a concentration of nanosilver from 5 to 0.003 ppm. Solutions of microorganisms in Muller-Hinton broth with a higher (10^8 CFU ml^{-1}) and lower (10^5 CFU ml^{-1}) cell number were prepared. To each well 180 µl of microbial suspension and 20 µl of serial dilutions of the specimen were introduced. As a negative control a Mueller-Hinton medium was applied and as a positive control a suspension of the microorganism with an addition of water was used. Plates were incubated in 30 or 37 °C and the optical density was measured at a wavelength of 600 nm every hour for 24 hours. Results were demonstrated as a minimal inhibitory concentration needed to cause as least 80% of growth inhibition and as a curve of growth.

Evaluation of antimicrobial properties of linen socks with the NANOfresh specimen and cotton socks containing silver (Silver SeaCell socks)

Both types of socks were cut into pieces of 2 cm². All pieces of linen sock were sterilised in autoclave and then immersed in 1 ml of the NANOfresh specimen to absorb the liquid. After the absorption of 0.5 ml of the specimen, pieces of socks were left to dry for 24 hours. With Silver SeaCell socks, half of the pieces were sterilised to obtain sterile material and better observation of the growth of indicator microorganisms. Next the pieces of socks were inoculated with the suspensions of microorganisms prepared (10^4 CFU ml^{-1}) in a quantity 100 µl and 200 µl. The control samples were pieces of socks immersed in sterile saline inoculated with the same amount of bacteria suspension.

The samples (for both types of socks) were then incubated in a temperature of 30 or 37 °C depending on the microorganism. After 24 hours, the pieces of socks were put into flasks with sterile saline and shook. The cell number was determined by the plating method using an appropriate medium. The antimicrobial activity was expressed as a percentage reduction of the cell number in comparison with the control sample.

Results and discussion

Evaluation of antimicrobial properties of the NANOfresh specimen

To evaluate the antimicrobial efficiency of the NANOfresh specimen, the activity spectrum and minimal inhibitory concentration on the microorganisms tested was determined. Results of these experiments are presented in ***Table 1***.

Results showed that tested preparation has a broad activity spectrum and inhibits the growth of different types of microorganisms, although the susceptibility of bacteria and yeasts examined was varied. Generally bacteria were more sensitive than *Candida albicans* yeasts, with the most sensitive species being *Staphylococcus epidermidis*. Inhibition of the growth of *S. epidermidis* was observed at a concentration of nanosilver amounting 0.125 ppm. Minimal inhibitory concentration did not depend on the cell density used, although growth curves were slightly different (***Figure 1***). The same dilutions of the specimen showed antimicrobial activity in both cases but the percentage of growth reduction was higher when the level of cell density was 10^5 CFU ml^{-1}. For example, at a concentration of 0.004 ppm nanosilver (0.78% of specimen) 53% growth reduction was observed when the initial density was established at a level 10^5 CFU ml^{-1}, while 40% growth reduction was noted in the case of cell density 10^8 CFU ml^{-1}. This experiment showed that a relationship between the amount of microorganisms and the concentration of silver nanoparticles is crucial for a final reduction of microorganisms.

Data in ***Figure 1*** demonstrate that, usually, differences between the control trial and samples with an addition of the specimen containing nanosilver were observed after three hours of incubation. Similar results were observed for other species examined. Effective control of all microorganisms tested needed at least 0.5 ppm of silver nanoparticles; however, in the case of *B. megaterium* and *C. albicans* this amount did not cause 80% growth in-

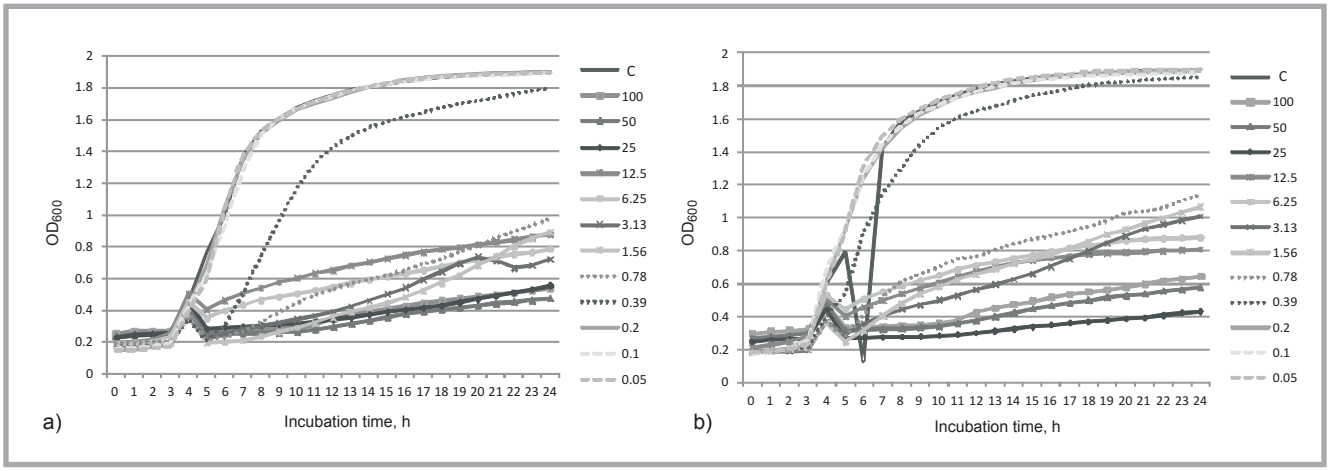

Figure 1. *Effect of serial dilutions of the NANOfresh specimen on the growth of Staphylococcus epidermidis. a) initial density of culture at a level 10^5 CFU ml^{-1},b) initial density of culture at a level 10^8 CFU ml^{-1} (numbers in legend indicate the dilution of specimen added).*

hibition. It was probably caused by the ability of *B. megaterium* to form spores, which have a different structure to vegetative cells. Spores have thick covers and are resistant to many substances. Therefore penetration of silver nanoparticles could be limited through a cover. Also the structure of the yeast cell wall is completely different in comparison with the bacterial cell envelope, which can be a reason for higher resistance to antimicrobial agents.

Data in literature confirm the differentiated activity of silver nanoparticles against microorganisms. For example, Wasif and Laga [32] observed stronger activity of commercial products containing nanosilver applied on cotton towards Gram-positive than Gram-negative bacteria. Filipowska et al. [33] presented new method of incorporating silver into textiles and proved antibacterial and antifungal properties of the material prepared.

Antimicrobial effectiveness is strongly dependent on the concentration of silver nanoparticles, on their shape and size and even on the conditions, such as the pH of the environment [34 – 37]. Sathishkumar et al. [38] used *E. coli* as model bacteria to test the bactericidal effect of the nano-crystalline silver particles produced. They observed 79.6% and 99.1% growth inhibition with 25 and 50 mg l^{-1} of nanosilver, respectively, for bacteria grown. Shahrokh and Emtiazi [39] observed antimicrobial properties of nanosilver at low concentration. The growth of *S. aureus*, *Bacillus* sp. and *E. coli* was inhibited at 0,5, 1 and 2 ppm of nanosilver, respectively. Moreover, Ruparelia et al. [40] reported a different inhibitory effect of silver nanoparticles depending on the strains of the same species. Minimum inhibitory concentration amounted to 60 - 200 mg l^{-1} for various strains of *E. coli*. Authors assumed that this difference was due to the varied susceptibility of the organisms tested. In the study of Kim et al. [6] the antimicrobial activity of silver nanoparticles was assayed against yeast, *E. coli* and *S. aureus*. They obtained higher efficacy against *E. coli* and yeasts and lower efficacy for *S. aureus*.

Generally silver products are effective against bacteria but their antifungal effect has received only marginal attention. Panáček et al. [41] investigated the antifungal activity of silver nanoparticles prepared by the modified Tollens process against different species of *Candida*. Results revealed that the nanosilver showed an inhibitory effect at a concentration as low as 0.21 mg l^{-1} using non-stabilized nanoparticles and 0.05 mg l^{-1} using SDS-stabilised silver nanoparticles. However, fungicidal activity was considerably higher. Minimum fungicidal concentration amounted to 27 mg l^{-1} for non-stabilised and 3.38 mg l^{-1} for SDS-stabilised silver nanoparticles. The authors also observed the varied sensitivity of test strains of *Candida*. The lowest MIC of silver nanoparticles the authors obtained was against *Candida albicans* strains, while the least sensitive amongst the tested yeasts was *C. parapsilosis*. Panáček et al. [40] drew attention to the fact, that the fungicidal activity of silver nanoparticles is lower than bactericidal effects [42].

Table 2. *Microorganism growth on linen socks with the NANOfresh specimen.*

Sample		Amount of bacteria suspension, µl	Cell number, cfu/ml	Percent of growth reduction, %
Staphylococcus aureus	control sample	100	$7.1 \cdot 10^4$	42
	experimental sample		$6.7 \cdot 10^2$	
	control sample	200	$1.5 \cdot 10^5$	45
	experimental sample		$6.7 \cdot 10^2$	
Staphylococcus epidermidis	control sample	100	$7.1 \cdot 10^4$	98
	experimental sample		1.3	
	control sample	200	$3.2 \cdot 10^5$	99
	experimental sample		0.3	
Bacillus megaterium	control sample	100	$2.4 \cdot 10^4$	12
	experimental sample		$6.4 \cdot 10^3$	
	control sample	200	$6.8 \cdot 10^5$	35
	experimental sample		$5.8 \cdot 10^3$	
Pseudomonas aeruginosa	control sample	100	$7.9 \cdot 10^4$	25
	experimental sample		$5.1 \cdot 10^3$	
	control sample	200	$2.1 \cdot 10^5$	23
	experimental sample		$1.2 \cdot 10^4$	
Candida albicans	control sample	100	$12 \cdot 10^4$	50
	experimental sample		$1.1 \cdot 10^2$	
	control sample	200	$4.4 \cdot 10^5$	58
	experimental sample		$3 \cdot 10^3$	

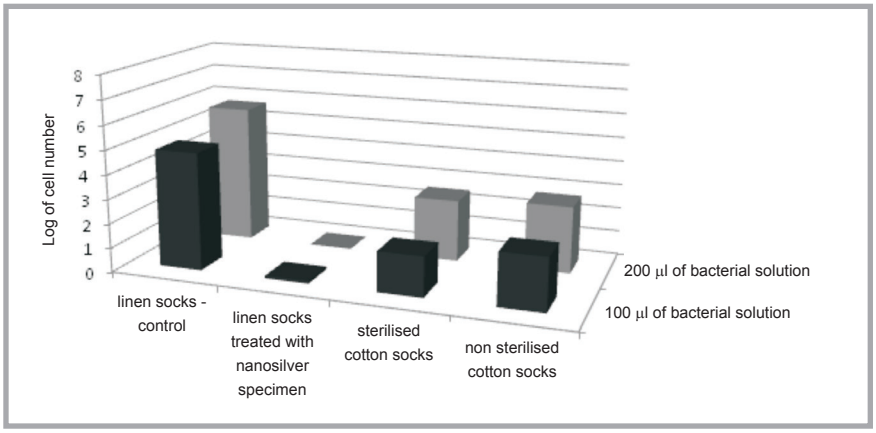

Figure 2. *Growth inhibition of bacteria S. epidermidis caused by cotton socks containing nanosilver in comparison with linen socks treated with the NANOfresh specimen.*

Concluding data in the literature as well as results in the paper presented indicates the varied activity of silver nanoparticles depending on many factors. As was shown in this paper, results may also be dependent on the method of antimicrobial assay.

Evaluation of antimicrobial properties of linen socks with the NANOfresh specimen

Based on the tests performed, it was concluded that the NANOfresh specimen applied to linen socks caused growth inhibition against the microorganisms tested. However, the degree of growth inhibition was varied, depending on the microorganism. In the next part of the investigation, the NANOfresh specimen was applied on pieces of linen socks and incubated with the microorganisms tested. According to the results shown in ***Table 2***, application of a specimen containing nanosilver caused a reduction in the cell number of all microorganisms tested. The highest effect of the NANOfresh specimen for growth inhibition was shown against bacteria *S. epidermidis*. Bacteria *S. aureus*, *P. aeruginosa* and fungi *C. albicans* were less sensitive to the NANOfresh specimen. The effect was observed for both 100 and 200 µl of bacteria suspension applied to the material. Except for *B. megaterium*, the degree of growth reduction was similar for both microbial solutions examined. In the case of bacteria *B. megaterium* the degree of growth inhibition was higher after application of 200 µl of bacteria suspension; however, the final number of cells was similar for both suspensions, reaching 5.8 to 6.4×10^3 CFU ml^{-1}.

Evaluation of antimicrobial properties of cotton socks containing nanosilver

Tests of antimicrobial properties of cotton socks containing silver were conducted on bacteria *Staphylococcus epidermidis*, which were the most sensitive to silver in the experiment described above. Sock pieces were divided into two parts and one was sterilised to obtain material free of microorganisms to avoid possible false positive results. All pieces were inoculated with a solution of *S. epidermidis* and incubated in optimal conditions for 24 h. Then the number of bacteria was determined as described earlier. Because of a lack of the same type of socks without nanosilver as control trials, linen socks inoculated with the same solutions of bacteria were used. Results were also compared with linen socks treated with the nanosilver specimen (***Figure 2***).

After application of bacteria onto the surface of cotton socks in the same amount as on the surface of linen socks, a reduction in the number of bacteria was observed both for sterilised and non sterilised pieces. For sterilized socks the cell number was $4.5\cdot10^1$ CFU ml^{-1} for 100 µl of bacteria suspension applied to material and $3.7\cdot10^2$ CFU ml^{-1} for 200 µl of bacteria suspension. For non sterilised socks the results were $1.5\cdot10^2$ CFU ml^{-1} and $5.7\cdot10^2$ CFU ml^{-1}, respectively. Higher amounts of bacteria in the case of non sterilised socks were probably the result of the presence of some saprophytic bacteria in the samples (data not shown), which could not be distinguished from *S. epidermidis* because of the use of a non-selective medium.

Data in literature confirm the possibility of using nanosilver as an antimicrobial agent in different types of materials such as textiles applied both on the surface and incorporated into the fabrics. Wasif and Laga [32] treated cotton woven fabric with some commercial products containing nanosilver and observed the antibacterial properties of such materials prepared against *Staphylococcus aureus* and *Escherichia coli*. They stated that the higher the concentration of the antimicrobial agent, the larger the zones of inhibition noted. Similarly the excellent antibacterial activity of silver nanoparticles on cotton fabric against *Staphylococcus aureus, Staphylococcus aureus methicillin resistance strain (MRSA), Escherichia coli,* and *Pseudomonas aeruginosa* was noted by Saengkiettiyut et al. [43]. Matyjas-Zgondek et al [36] investigated the bacteriostatic activity of selected silver particles: nano-Ag, sub-micro-Ag and AgCl in the finishing of textiles against *Bacillus subtilis* and *Escherichia coli*. Results showed strong antibacterial activity of all silver compounds used; however, the antimicrobial effectiveness was strongly dependent on the concentration of silver in the finishing bath and type of particles. Mahltig and Haase [44] compared the antibacterial effect of six silver-containing textile products against *Escherichia coli* and *Staphylococcus aureus*. Four of them were textiles commercially distributed in Europe; one product was supplied as a liquid agent for application in household washing machines, and the last sample was a viscose fabric coated with a solution containing silver particles stabilised with polypyrollidone. The antimicrobial effects varied depending on the type of fabric, but were comparable for both bacteria tested. The best results were obtained for nanosilver multipurpose cloth and antiseptic socks, while the product designed for application during washing was nearly ineffective. The authors also stated that some commercialised silver fabrics did not show the antimicrobial activity as promised.

Conclusions

Silver nanoparticles have an increased interest due to their antimicrobial activity and are incorporated into different products such as textiles. The presented work demonstrates antimicrobial properties of linen socks impregnated with silver nanoparticles specimen with comparison to the commercial cotton socks containing nanosilver.

Linen socks treated with the NANOfresh specimen exhibited the highest antimicrobial activity against bacteria *S. epidermidis,* followed by bacteria *S. aureus* and *P. aeruginosa*. Less sensitive were spore-forming bacteria *B. megaterium* and *C. albicans* yeasts. It was also found that the growth inhibition of bacteria *S. epidermidis* was higher for linen socks with the NANOfresh specimen than for Silver SeaCell socks (both sterilised and non sterilized). An explanation for the results obtained may be the fact that according to the producer, nanosilver concentration in Silver SeaCell socks was 4 ppm, which was slightly lower than in linen socks with the NANOfresh specimen, which was 5 ppm. Taking into account minimal inhibition concentration for the specimen tested, it is possible that 4 ppm of silver nanoparticles did not inhibit the growth of the bacteria examined as efficiently as with the NANOfresh preparation. Another reason could also be the fact that after application of the specimen on socks, probably the majority of silver nanoparticles were on the surface and therefore their contact with microbial cells could be easier. It is worth noting that very important is the number of microbial cells in contact with nanosilver. The socks tested differed also in their composition. According to the manufacturer of Silver SeaCell socks, due to the presence of algae in the yarn, socks made of it contain minerals, vitamins, carbohydrates and amino acids. These compounds are known to provide an appropriate environment for microbial growth, which indicates that the socks' composition could influence *S. epidermidis* bacteria growth, which was higher in Silver SeaCell socks than in linen socks with the NANOfresh specimen.

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EPNOE 2013

3rd EPNOE International Polysaccharide Conference

POLYSACCHARIDES AND POLYSACCHARIDE - DERIVED PRODUCTS FROM BASIC SCIENCE TO APPLICATIONS

Nice (France)

21-24 October 2013

The European Polysaccharide Network of Excellence (EPNOE) and the Cellulose and Renewable Materials Division of the American Chemical Society (ACS) join their efforts in promoting basic and applied sciences of polysaccharides by organising common events, one of which is the 3rd EPNOE conference.

This conference is a forum bringing together academic, industrial, and government scientists and students dealing with polysaccharides and polysaccharide-derived products. Many specialists including biologists, chemists, physicists, food specialists, technologists, and environmentalists will meet and gain knowledge about the interdisciplinary world of polysaccharide science.

All research and application topics related to polysaccharides are within the focus of EPNOE 2013, particularly:

- Polysaccharide isolation and characterisation (algae, new crops and plants, by-products, wastes, other sources)
- Biosynthesis of polysaccharides
- Biodegradation of polysaccharides (mechanisms, products determination, efficiency), enzymology
- Chemical modification of polysaccharides
- Advanced physical, chemical, structural, and surface characterisation of polysaccharides
- Nanotechnology: nano- and micro- polysaccharide-based objects (production, characterisation, use)
- Biomimetic applications of polysaccharides
- Development of fuels based on polysaccharides
- Polysaccharides and food ingredients
- Polysaccharides for biomedical applications
- Polysaccharide-based materials including films, fibres and composites
- Bioplastics
- Pulp and paper
- Life Cycle Assessments- Environmental concerns –Policy – Social aspects
- New trends on polysaccharide research and applications (session organised by PhD students and post-doctoral researchers from EPNOE partners).

A round-table discussion on education, an evening session for the general public and a special session entitled “Polysaccharide-based bio-economy: visions of CEOs and major stakehoders” are planned. In addition to oral and poster presentations, two special guests, four plenary lecturers and more than 30 invited lecturers from all over the world will deliver a speech.

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