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Optical methods for evaluating the composition of combined materials based on bacterial biocellulose

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The results of investigating the composition of bacterial cellulose (BC)-based composite materials using Raman spectroscopy (RS) and confocal fluorescence microscopy method are provided. Based on the variance analysis, an algorithm was developed to identify the investigated BC-based materials using a decision tree. The research results were confirmed by microscopic analysis.

Keywords: Bacterial biocellulose, Raman spectroscopy, microscopic analysis, Raman spectra, identification algorithm.

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Introduction

A growing demand for bio-based bacterial cellulose materials makes their application in biomedical fields increasingly appealing. Bacterial cellulose (BC)-based combination materials promote re-epithelialisation by stimulating cell adhesion, proliferation, migration and differentiation, resulting in faster wound healing in regenerative medicine [1]. At the same time, the survivability of such biomaterials directly depends on their composition. Therefore, controling the composition of the combined materials based on bacterial biocellulose during their manufacture is a pressing issue.

Such rapid, non-invasive optical methods of analysing biomaterials as microscopic analysis [2], infrared spectroscopy [3] and Raman spectroscopy have been widely used in biomedicine [4].

The literature lacks information on the study of BC-based combined materials and their analysis using a complex of optical research methods.

This study aims to apply a set of optical methods to evaluate the composition of bacterial biocellulose-based combined materials.

Materials and methods

In this paper, bacterial cellulose (BC) and combined materials based on it including various additives such as pectin, 1.3-dioctyl-4-methylimidosaluminium bromide were investigated. Conventionally, all the studied samples were divided into 4 main groups: *1* — BC gel with pectin content; *2* — BC gel without pectin content; *3* — 1.3-dioctyl-4-methylimidosaliy bromide BC with added pectin; *4* — 1.3-dioctyl-4-methylimidosaliy bromide BC without added pectin.

To obtain BC, Acetobacter was cultured from Medusomycesgisevii culture by cloning with seeding from a dilution of 1:100000 on agarised HS medium (Hestrin Schramm, 1954). BC gels were washed with running water and placed in 0.1H NaOH solution for 24 hours at room temperature to remove any remaining bacterial cells. Washing with water was repeated and placed in 0.5% HCl solution also for 24 hours at room temperature, then washed again with running water, then distilled water to neutral pH value and stored in 70% ethyl alcohol in plastic dishes at 25−27°C. Gel-based composites were prepared by synthesising imidazolium derivatives. The gels were soaked in 250 μg/ml DMSO solutions of imidazolium derivatives for at least 24 h and further used to investigate the antibiotic properties.

The spectral characteristics of the investigated biomaterials were studied by Raman spectroscopy, which was implemented using the experimental setup described in detail in [5]. A laser with a wavelength of 785 nm was used. The resolution of the spectrum was 1.5 cm^{-1}. The mathematical processing of the RS spectra was carried out with the RS Tool software using linear variance analysis.

The method of confocal microscopy was implemented using a stand based on an Olympus IX 71 confocal optical microscope (Japan) and an ANDOR laser engine. The bench provided two modes of operation: confocal microscopy in visible light and laser fluorescence mode. A halogen lamp was used as the radiation source [6].

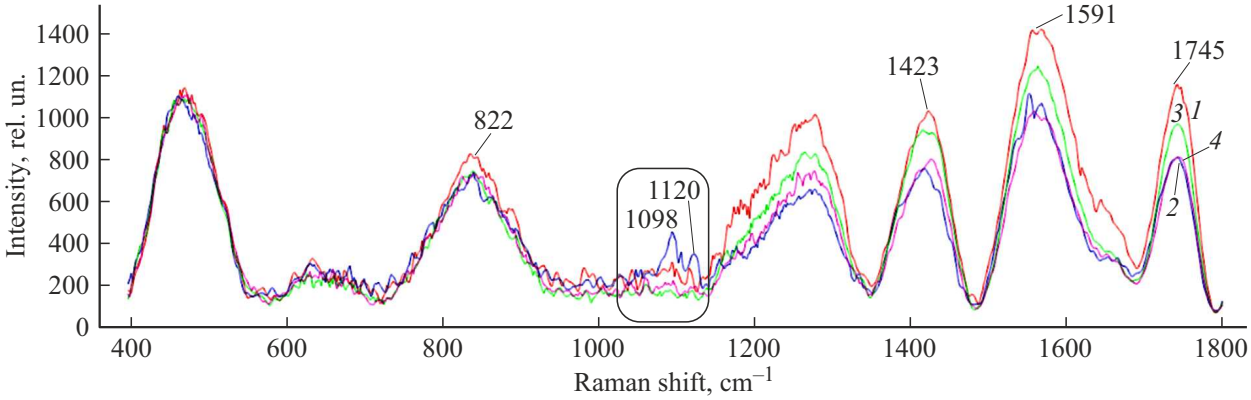

Figure 1. Averaged RS spectra of the investigated groups (spectra were averaged within all group spectra): groups *1* (red line) and *2* (blue line), groups *3* (green line) and *4* (purple line).

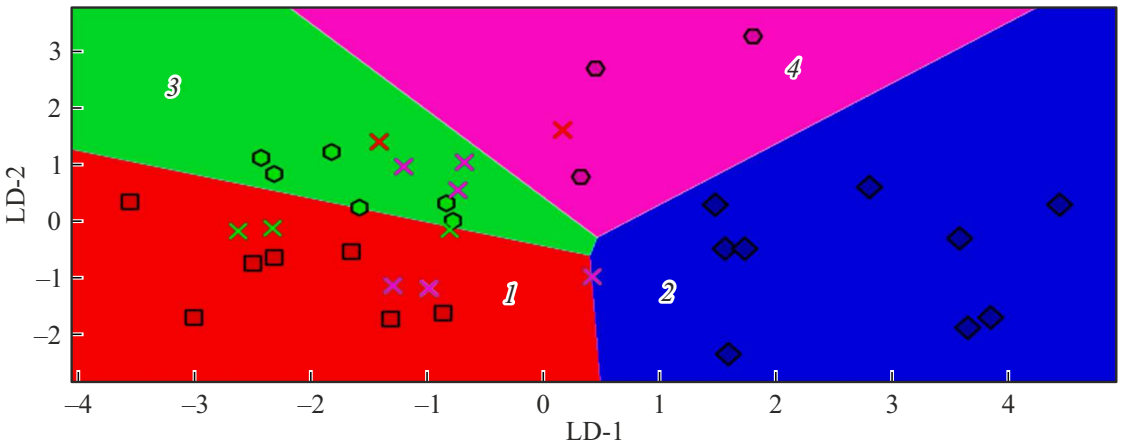

Figure 2. LDA results. Linear discriminant function value plot (*1, 2, 3* and *4* — study groups).

Results

The figure shows the averaged RS spectra of the investigated groups (the spectra were averaged within all group spectra).

The analysis of Fig. 1 shows that in the spectral range from 800–1800 cm^{-1} there are changes in the intensity of the main RS lines characteristic of biocellulose. It can be seen that the 822 cm^{-1}, line corresponding to pectin is determined on the RS spectra in groups *1, 3* (groups with pectin content). RS lines ~ 1098, 1120, 1180–1275, 1423 and 1745 cm^{-1}, corresponding to cellulose vibrations, are most pronounced in BC samples without added pectin, which is probably due to the fact that the ratio of the RS lines corresponding to pectin and cellulose varies depending on the composition of the studied groups. Moreover, the RS line ~ 1098 cm^{-1} is detected only in BC samples without pectin content. This line is rather highly sensitive to the orientation of microfibrils along the fibre, so it has a property to stretch in amplitude on the given spectra.

Further mathematical processing was carried out using LDA analysis and a decision tree in RS-Tool program. To increase the information content of the obtained Raman spectra, a linear variance analysis of the spectra was conducted, which included their decomposition into spectral lines. During the simulation, the line intensity was selected from 0 to the local maximum of the spectrum in the x_0 region. The variable a was a criterion for line amplitude, which depends on the values of the independent regressors dx and x_0, which determine the initial analysis conditions. The average value of the spectrum determination coefficient from the initial one in the region 400–1800 cm^{-1} for all 74 spectra was $R^2 = 0.99$. The calculated accuracy = 92.0%, precisionscore = 36.36%. The discriminatory adequacy of the method is characterised by the AUC = 0.95 value.

Fig. 2 shows the results of LDA comparison of the sample groups. The discriminant function LD-1 describes a variance of 77–74%. Positive values of LD-1 are typical of the RS spectra of bacterial cellulose gel samples without pectin content as well as 1.3-dioctyl-4-methylimidosalic bromide BC without added pectin. The regions of groups *2* and *4*

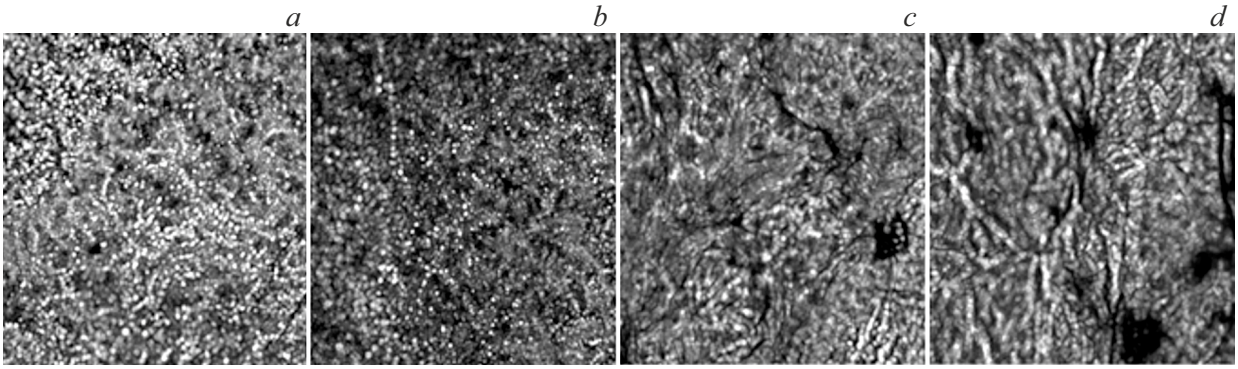

Figure 3. Micrographs of bacterial cellulose (*a* — group *1*, *b* — group *2*, *c* — group *3*, *d* — group *4*) obtained by confocal microscopy. The field size is $400 \times 400\,\mu$m.

have a single intersection ~ 0.5 of Fig. 2. The regions of groups *1* and *3* have areas of intersection in ~ -0.87 of Fig. 2. The studied groups with and without pectin are distinguished using LDA analysis. The main differences between the investigated groups are defined by the following main RS lines: ~ 1745, 1591 and $1092\,\text{cm}^{-1}$.

Taking into account the variance analysis and using two of the above-mentioned RS lines, on which the main differences between the studied groups are observed, the following decision tree was created: if the amplitude of the RS line $\sim 1092\,\text{cm}^{-1}$ is below 0.075 rel. units, it is the spectrum of BC without pectin content. If the amplitude of the RS line $\sim 1277\,\text{cm}^{-1}$ is 0.075 rel. units, it is the BC spectrum with pectin content. If the amplitude of RS line $\sim 1763\,\text{cm}^{-1}$ line is lower than 0.018 rel. units, it is the BC spectrum with 1.3-dioctyl-4-methylimidosalic bromide with pectin addition, and if the amplitude of RS line $\sim 1745\,\text{cm}^{-1}$ is 0.018 rel. units, it is the BC spectrum without pectin content.

Further, Fig. 3 shows the results of microscopic analysis of the investigated objects.

Fig. 3 shows micrographs of dried BC gel samples with added pectin and 1.3-dioctyl-4-methylimidosaluminium bromide. The addition of pectin led to the formation of larger BC granules. The samples have a granular structure, with the addition of the imidosalium derivative leading to partial ordering, arranging the BC granules into chains, which can be clearly seen in Fig. 3.

Conclusions

Using Raman spectroscopy and LDA, as well as variance analysis, spectral differences between the BC groups studied with and without the addition of pectin and with the addition of 1.3-dioctyl-4-methylimidosalium bromide were identified. It was found that the main differences in the RS spectra are manifested on the lines $1745\,\text{cm}^{-1}$, $1591\,\text{cm}^{-1}$ and $1092\,\text{cm}^{-1}$corresponding to the main lines of bacterial cellulose.

An algorithm for the identification of BC-based objects was developed. It was found that if the amplitude of the RS line $\sim 1745\,\text{cm}^{-1}$ is 0.018 rel. units, it is a BC spectrum without pectin content, while if the amplitude of the line $1277\,\text{cm}^{-1}$ is 0.075 rel. units, it is a BC spectrum with pectin content.

Thus, it is possible to perform an express analysis of the composition of the studied samples based on the bacterial cellulose using the Raman spectroscopy method.

Microscopic analysis revealed changes in BC structure upon addition of pectin in all the samples studied.

Conflict of interest

The authors declare that they have no conflict of interest.

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