The hexahistidine containing organophosphorus hydrolase enzyme and bacterial cellulose based functional materials

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Abstract. Bacterial cellulose (BC) is a unique biopolymer for creation on its basis functional materials used as drugs, matrices for regenerative therapy, dressings, etc. In this study, the possibility of combining of BC with hexahistidine containing organophosphorus hydrolase (His₆-OPH), exhibiting lactonase activity against gram-negative pathogenic bacteria is considered to obtain composite materials with special properties. The dry and wet samples of fibrous BC, impregnated with solutions of the His₆-OPH enzyme and various antimicrobial agents were obtained; their catalytic characteristics were investigated. The preservation of the enzymatic activity of the His₆-OPH enzyme in the composition of the obtained samples with BC, including after the lyophilization process, has been established. The stabilization of the enzymatic activity of His₆-OPH in the composition of fibrous materials in the presence of poly(aspartic) acid and antimicrobial agents is shown.

1. Introduction
Bacterial cellulose (BC) is of particular interest as a biocompatible material for scientific research. BC is a fibrous biopolymer synthesized by Acetobacter xylinum cells and some other types of bacteria [1]. BC does not contain impurities of hemicellulose or lignin and does not require cleaning in contrast to plant cellulose. Due to this, the process of BC obtaining is significantly simplified, and BC molecules form a nanofibrillar structure. In this process, BC has high strength characteristics and crystallinity degree, which can reach 90%, good light transmittance factor, biocompatibility, cell adhesion, and is also characterized by high permeability to liquids and gases, which allows using this fibrous material to create various modern composite materials for different purposes [2]. Due to its unique properties, BC is widely used in such industries as water purification, cosmetic, the preparation of sensor technologies, etc. [3, 4, 5]. The BC is especially widely used in medicine as surface coatings for implants, dressings, etc. [6, 7]. It is known that BC is actively used as components for fibrous and other composite materials. For example, it was shown that when poly(vinyl) alcohol (PVA) cryogels are filled with BC nanofibers, the rigidity and heat resistance of the composites increase in comparison with unfilled samples. Nanomaterials containing particles of gold, silver and zinc oxide with bactericidal and conductive properties have already been developed on the basis of BC [8].
In this study an attempt was made to create a fibrous material using an enzyme such as hexahistidine containing organophosphorus hydrolase (His\textsubscript{6}-OPH) as a partner for combining with BC. It is known that the His\textsubscript{6}-OPH enzyme has a high catalytic activity in the processes of hydrolysis of a number of toxic organophosphorus compounds, and also exhibits lactonase activity against a number of signaling molecules of pathogenic gram-negative bacteria - N-acyl homoserine lactones (AHLs) [9, 10].

Since in recent years there has been a rapid increase in the number of antibiotic-resistant microorganisms and a significant decrease in the efficiency of many of the known antibiotics, it is necessary to find an alternative to these antibiotics [11]. For this purpose, antimicrobial preparations based on the His\textsubscript{6}-OPH enzyme or its poly(electrolyte) complexes (PEC) and various antibiotics were obtained earlier, and their catalytic characteristics were studied [12, 13]. It has been established that antibiotics do not have a measurable negative impact on the His\textsubscript{6}-OPH enzyme, and their enzyme inhibitory concentrations do not exceed the minimum inhibitory concentrations of antibiotics applied to bacterial cells. It also showed that the combination of the His\textsubscript{6}-OPH enzyme and β-lactam antibiotics leads to the stabilization of the catalytic activity of His\textsubscript{6}-OPH and an increase in the action efficiency of both the enzyme and antibiotics. These results were also confirmed by molecular docking method [13].

The objective of this paper was to assess the possibility of obtaining and studying the catalytic characteristics of biocompatible functional materials based on BC and the His\textsubscript{6}-OPH enzyme containing various antibacterial agents for the manifestation of antimicrobial properties and the main catalytic activity of the enzyme itself.

2. Results and Discussion

The dry and wet samples of BC were exposed in distilled water for a certain time at T = 8 °C. The mass of samples with BC nanofibers was measured periodically until the termination in weight change (Figure 1).

![Figure 1](image)

**Figure 1.** The swelling kinetic of dry (a) and wet (b) samples with BC, where ♦ - BC+His\textsubscript{6}-OPH/meropenem/Ag; ▼ - BC+His\textsubscript{6}-OPH/meropenem; ○ - BC+His\textsubscript{6}-OPH/PLD/Ag and □ - meropenem.

It has been shown that the highest rate of swelling is observed in the case of dry sample with BC, as expected. At the same time, in both cases of dry and wet samples with BC, the swelling rate of the sample BC + His\textsubscript{6}-OPH/meropenem/Ag was maximized. It should be noted that different kinetic of swelling was observed for the remaining samples, in the case of dry and wet samples of BC, which may be explained by the difference in the porosity of dry and wet BC nanofibers.
8 samples of different composition based on both dry and wet BC nanofibers were obtained to study their catalytic characteristics (Table 1).

**Table 1.** Composition of samples based on BC nanofibers.

| #  | Composition                  | Antimicrobial agent |
|----|-------------------------------|---------------------|
| 1  | His<sub>6</sub>-OPH          | none                |
| 2  | His<sub>6</sub>-OPH          | meropenem           |
| 3  | His<sub>6</sub>-OPH          | Ag                  |
| 4  | His<sub>6</sub>-OPH          | meropenem + Ag      |
| 5  | His<sub>6</sub>-OPH/PLD      | none                |
| 6  | His<sub>6</sub>-OPH/PLD      | meropenem           |
| 7  | His<sub>6</sub>-OPH/PLD      | Ag                  |
| 8  | His<sub>6</sub>-OPH/PLD      | meropenem + Ag      |

The obtained samples were exposed for 16 hours at T = 8ºC and then were lyophilized. The enzymatic activity of His<sub>6</sub>-OPH in the composition of the obtained samples was determined before and after drying (Figure 2).

As a result, it has been found that there is a specific increase in the enzymatic activity of His<sub>6</sub>-OPH in all BC samples, after drying. For samples with dry BC nanofibers, higher values of His<sub>6</sub>-OPH activity were observed in comparison with wet samples of BC. As noted above, this may be due to the different porosity of dry and wet BC nanofibers, which in turn leads to differences in carrier capacity. As a result, a higher capacity of the dry BC fiber leads to better absorption of enzyme solutions and, therefore, greater enzymatic activity.

A higher enzymatic activity of samples containing His<sub>6</sub>-OPH/Ag than with His<sub>6</sub>-OPH/meropenem/Ag or His<sub>6</sub>-OPH/meropenem was noted. At the same time, there was no difference in the effect of antimicrobial agents on the enzymatic activity for samples containing PEC of His<sub>6</sub>-OPH with poly-(L-aspartic acid). However, in the case of dry BC, the enzymatic activity of samples with the pure His<sub>6</sub>-OPH enzyme was lower than with its PEC in the presence of antimicrobial agents.

### 3. Conclusions

The results of the study showed that composite materials with stable enzymatic activity and potential antimicrobial properties can be obtained on the basis of bacterial cellulose and the His<sub>6</sub>-OPH enzyme.
The use of the enzyme-poly(electrolyte) complex in the presence of antimicrobial agents contributes to a better stabilization of the His\textsubscript{6}-OPH enzymatic activity.

4. Materials & Methods

4.1. Materials
The poly-(L-aspartic acid) sodium salt (PLD\textsubscript{50}, MW = 6.8 kg\textsuperscript{-1}mol\textsuperscript{-1}) (Alamanda Polymers, Huntsville, AL, USA) was used for production of enzyme polyelectrolyte complexe. Antibiotic (meropenem) was acquired in the local pharmacy and was used as received. Paraoxon and other reagents were purchased from Sigma (Saint Louis, MO, USA). Recombinant \textit{Escherichia coli} strain SG13009[pREP4] (Qiagen, Hilden, Germany) transformed by plasmid encoding His\textsubscript{6}-OPH [14] was used for His\textsubscript{6}-OPH production.

4.2. Preparation of enzyme
Recombinant cells of \textit{Escherichia coli} SG13009[pREP4] were cultivated, and the enzyme was isolated and purified by methods published previously [15]. The purified preparation of His\textsubscript{6}-OPH was characterized by enzymatic activity as described earlier [16]. The concentration of protein was determined by Bradford assay, and protein purity was analyzed by SDS-PAGE in 12% polyacrylamide gel using Mini-PROTEAN II cell (Bio-Rad, Hercules, CA, USA) followed by Coomassie Blue (R-250) staining. According to SDS-PAGE (data not shown), the purity of His\textsubscript{6}-OPH preparation obtained (MW \approx 37 kg\textsuperscript{-1}mol\textsuperscript{-1}) was ca. 98%. The specific organophosphorus hydrolase activity of the purified enzyme was 4300 U\textsuperscript{-1}mg\textsuperscript{-1}. One unit of enzymatic activity (U) was defined as the quantity of the enzyme necessary to hydrolyze 1 \mu mol of paraaxon per min at 25ºC.

Enzyme-polyelectrolyte complexe (EPC) was produced similarly to the known procedure [17]. In brief, to produce an His\textsubscript{6}-OPH/PLD\textsubscript{50}, an aliquot of 20 g\textsuperscript{-1}L\textsuperscript{-1} PLD\textsubscript{50} water solution was added to the purified His\textsubscript{6}-OPH (1.6 ± 0.1 g\textsuperscript{-1}L\textsuperscript{-1}) in 50 mM phosphate buffer (pH 7.5) containing 150 mM NaCl. The aliquot volume was calculated so that “enzyme:polymer” molar ratio was 1:5. Next, the mixture was held for 30 min at 8ºC. The effective hydrodynamic diameter of the particles of prepared complexe was determined at 25ºC by dynamic light scattering using a Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, UK) as it was published previously [17] of necessity.

4.3. Measurement of swelling kinetic
The BC film was produced and purified according to the procedure described earlier [18]. After the BC-film was washed in distilled water, its properties were investigated. Initial solutions of meropenem at concentration of 2 g\textsuperscript{-1}L\textsuperscript{-1} (5.2 mM) in 50 mM phosphate buffer (pH 8.0) containing 150 mM NaCl, and 60 mg\textsuperscript{-1}L\textsuperscript{-1} colloidal silver nanoparticles were mixed with the 2 g\textsuperscript{-1}L\textsuperscript{-1} solution of enzyme (28 \mu M) 1:1 volumetric proportion and exposed for 30 min at room temperature. Then the mixture was added to BC pellicles and exposed at temperature 8ºC for 48 h. The mass of the BC samples was periodically measured until the termination in its mass changes.

4.4. Measurement of enzyme activity
Initial solutions of meropenem, colloidal silver nanoparticles were mixed with the enzyme or EPC as described above and exposed for 30 min at room temperature. Then the mixture was added to BC pellicles and exposed at temperature 8ºC for 16 h. Thereupon the samples were lyophilized. The organophosphorus hydrolase activity was determined before and after lyophilization as described earlier [19]. The required concentration of a Paraoxon was added to a glass container with a batch of the BC sample and 0.1 M carbonate buffer (pH 10.5). Aliquots were removed from the container at regular intervals and analyzed using an Agilent-UV spectrophotometer equipped with a thermostated analytical cell to study the accumulation of 4-nitrophenolate anion at 405 nm. Enzyme concentration hydrolyzing 1 \mu mol of the substrate per minute at 25ºC was taken as the unit of enzymatic activity.
The rates of the enzymatic reaction were calculated by the initial linear sections of kinetic curves ($v_0 = tg\alpha$).

The every data are presented as means of at least three independent experiments ± standard deviation (±SD).

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