Plackett–Burman experimental design for bacterial cellulose–silica composites synthesis

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ABSTRACT

Bacterial cellulose–silica hybrid composites were prepared starting from wet bacterial cellulose (BC) membranes using Stöber reaction. The structure and surface morphology of hybrid composites were examined by FTIR and SEM. The SEM pictures revealed that the silica particles are attached to BC fibrils and are well dispersed in the BC matrix. The influence of silica particles upon BC crystallinity was studied using XRD analysis. Thermogravimetric (TG) analysis showed that the composites are stable up to 300 °C. A Plackett–Burman design was applied in order to investigate the influence of process parameters upon silica particle sizes and silica content of BC–silica composites. The statistical model predicted that it is possible for silica particles size to vary the synthesis parameters in order to obtain silica particles deposited on BC membranes in the range from 34.5 to 500 nm, the significant parameters being ammonia concentration, reaction time and temperature. The silica content also varies depending on process parameters, the statistical model predicting that the most influential parameters are water–tetraethoxysilane (TEOS) ratio and reaction temperature. The antimicrobial behavior on Staphylococcus aureus of BC–silica composites functionalized with usnic acid (UA) was also studied, in order to create improved surfaces with antiadherence and anti-biofilm properties.

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1. Introduction

Hybrid inorganic–organic composites are versatile materials with huge application areas from regenerative medicine to industry. The organic material is often a synthetic polymer, the use of biopolymers being more advantageous because of their biodegradability and biocompatibility, despite their higher cost. The interaction between the organic material and the inorganic substrate may have synergetic effects, which leads to the development of hybrid materials with improved properties [1,2]. Hybrid polymer/inorganic materials were also used as efficient bio-active surfaces, being suitable for the development of stabilizing and controlled release nanosystems [3–5]. Despite their proved biomedical potential, especially in infection control, several natural products, as highly volatile essential oils and their major fractions (eugenol, limonene, usnic acid etc.) fail to manifest their full potential due to their low volatility and high volatility, which leads to the necessity of being used in high therapeutic doses, which are usually toxic [6–8]. Usnic acid (UA) is one of such compound, very efficient to eradicate bacterial infections, especially with Gram positive etiology and it may be considered as an efficient alternative in the therapy of Staphylococcus aureus infections [9]. However, studies reported that this efficient antimicrobial compound has also a significant citotoxicity in vitro when it is used at antibacterial doses [9]. For this instance, developing novel, biocompatible and biodegradable nanometric polymeric shuttles, which may be used in increasing the efficiency of this compound at lower doses, thus reducing its citotoxicity, represent a current focus.

Among the numerous candidates for hybrid inorganic–organic composites biopolymer/silica nanocomposites were already reported as being suitable for the design of membranes and coatings, for the entrapment of bioactive molecules (like enzyme, drugs, cell markers) or for biosensors and bioreactors [10–14].

From the biopolymer class, cellulose and bacterial cellulose are also versatile materials which could be used to obtain inorganic–organic composites. Until now many composite cellulose–silica composites were reported using different types of cellulose and different synthesis methods [15–19]. Bacterial cellulose (BC), a well-known microbial polymer, could offer more possibilities to obtain biopolymer/silica composites, due to its superior properties in comparison with cellulose from plants, excepting its higher price. Exhibiting an ultrafine network structure and porous channels, BC could be used as a suitable template for in situ preparation of metal oxides through the sol–gel reaction [20–23].
BC–silica composites were also obtained using the solution impregnation method. Even if this method could improve the mechanical properties of the obtained composites, it is difficult to load BC sheets with more than 10% silica [24–26].

Among the silica synthesis methods, Stöber synthesis is very popular, because it leads to spherical and narrow dispersed silica micro and nanoparticles and because it is also considered simple by involving hydrolysis followed by polycondensation of the precursor under alkaline conditions [27,28]. Thereby, this method is intensively studied and the influence of the working parameters on the size and particle distribution is well known. For example, the most effective parameters are: concentration of tetraethoxysilane (TEOS), ammonia and water, polarity of the solvent and the reaction temperature [29–31].

When many quantitative and qualitative variables are involved in a process, statistical experimental design methods are widely used for optimization studies. Among these statistical methods, Plackett–Burman screening design is extremely useful in preliminary studies in order to identify the most important factors that could influence a studied phenomenon. The main advantage of this experimental design is to offer the possibility to screen a large number of parameters using a relative small number of experiments. The model doesn't describe the interaction between the parameters, because it is based on a first-order polynomial model [32].

To our knowledge the Stöber reaction hasn't been used to prepare BC–silica composite membranes and also Plackett–Burman experimental design hasn't been used to appreciate the influence of synthesis parameters upon silica particles' size and on silica content of BC–silica composites.

The aim of this study was to deposit silica on BC membranes by Stöber reaction, to characterize the influence of synthesis parameters on silica particles' size and silica content of BC–silica composites.

2. Materials and methods

2.1. Materials

Unless otherwise stated, all chemicals and reagents were supplied by Sigma-Aldrich and were used without further purification.

2.2. Production and purification of bacterial cellulose membranes

Bacterial cellulose membranes were produced by Gluconacetobacter sp. strain isolated from traditionally fermented apple vinegar in the Microbiology Laboratory of Chemical and Biochemical Engineering Department of University Politehnica of Bucharest. The culture was grown in a modified Hestrin–Schrämm (MHS) medium containing 2% fructose using a static culture at 28 °C for 7 days. The pellets obtained were purified by treatment with 0.5 N NaOH aqueous solutions at 90 °C for 1 h to eliminate the bacterial cells and then washed with deionized water until the pH of water became neutral. BC pellets were used as wet membranes.

2.3. Composite BC–silica membranes preparation

Tetraethoxysilane (TEOS) (98% analytical grade) was submitted to hydrolysis/condensation process in situ on the bacterial cellulose wet membranes. Bacterial cellulose pellet was immersed in an Erlenmeyer flask containing ethanol, distilled water and ammonium hydroxide in different concentration according with Plackett–Burman design. Finally, TEOS was added and the reaction was allowed to proceed for one or two hours at two different temperatures (respectively 20 °C and 50 °C) in accordance with factorial experiments. The BC–silica composites were then collected, washed with ethanol and distilled water and dried at 60 °C, over 24 h.

2.4. Composite BC–silica membranes as bioactive surfaces

2.4.1. Preparation

The preparation of bioactive surfaces was performed by coating a 1 cm² section of BC–silica membrane with usnic acid (UA). The layer of the usnic acid on the BC–silica sections was achieved by submerging the surfaces in 5 mL of phytofluid prepared from usnic acid and chloroform (UA/CHCl₃ 1% w/v), followed by extemporaneous drying at room temperature. The rapid drying was facilitated by the convenient volatility of chloroform. The coated surfaces were then sterilized by ultraviolet irradiation for 15 min, for further utilization in microbiological assay.

2.4.2. Bacterial adherence and biofilm formation assay

S. aureus ATCC 25922 strain was used to create an artificial biofilm. Adherence and biofilm formation were analyzed in 6 multi-well plates (Nunc), using a static model for monospecific biofilm development. Briefly, 1 cm² pieces of the tested materials were placed in 2 mL nutrient broth inoculated with 5 μL of an S. aureus suspension with a density of 0.5 McFarland. After 24 h of incubation, adherence was assessed by harvesting the adhered cells, following the viable cell count assay. The temporal dynamic of biofilm formation was studied by harvesting the biofilm developed on the BC–silica surfaces in nutritive broth at 24, 48 and 72 h. After incubation, the biofilm developed on the control and test surfaces was harvested and suspensions were performed in sterile PBS (phosphate saline buffer). The obtained suspensions were further ten-fold diluted and 5 μL of each dilution were seeded in triplicates on nutritive agar (BioMerieux), incubated for 24 h at 37 °C in order to determine the viable cell counts and thus, indirectly, to evaluate the number of viable bacterial cells embedded in biofilms. All experimental procedures were repeated three times and triplicates were used for each experiment. Fig. 1 presents the schematic illustration of expected biofilm development on the coated/uncoated BC–silica composites.

2.5. BC–silica membranes characterization

The BC–silica composites were examined on a Jasco FT/IR6200 spectrometer (AB& L-E-JASCO Romania) with Intron μ Infrared Microscope with ATR-1000-VZ objective. The spectra were the average of 50 scans recorded at a resolution of 4 cm⁻¹ in a range from 4000 to 500 cm⁻¹ with a TGS detector.

For morphological observations a scanning electron microscope HITACHI S-2600N (Hitachi Romania, Japan) was used, with resolution in secondary electron image 4.0 nm, electron gun with filament: tungsten hairpin type; accelerating voltage: 0.5–30 kV, emission current 10–12–10–7 A. The work conditions were: accelerating voltage: 25 kV, WD (working distance) 13 mm and beam 30, in a good agreement with the physical characteristics of the sample. All samples were gold coated prior to SEM examination.

The X-ray diffraction (XRD) measurements were conducted using a Shimadzu XRD 6000 diffractometer (Ni filtered Cu-Kα radiation, 40 kV, 30 mA and 0.02° step scan).

The thermal behavior of the composites was tested using thermogravimetric analysis on a thermal analyzer (DTG-60–Shimadzu). The operating conditions were: temperature range of 20 °C to 1000 °C, with a heating rate of 10 °C/min, and air flow rate of 50 mL/min.

The digital image analysis was carried out using IMAGE J 1.47f software, a program developed at the National Institute of Health of the USA and available on Internet (http://image.nih.gov/ij).

2.6. Water vapor transmission rate

Water vapor transmission rate (WVTR) was determined using a modified ASTM method (ASTM E 96-95, 1989). The test cups were filled
with 20 g of silica gel (desiccant) to produce a 0% RH below the film. Silica gels were heated at 180 °C for at least 8 h prior to use for the determination. The cups were placed at 30 °C in a desiccator containing distilled water and a temperature and humidity sensor. The cups were weighed periodically over a 192 h time interval. To WVTR values were determined, by linear regression, as the slopes of the steady state period of the curves of weight loss as a function of time for all the samples.

\[
\text{WVTR (g m}^{-2} \text{day}^{-1}) = \frac{J}{A}
\]

(1)

where: J is the slope of the weight loss curves versus time (g/day) and A is the area of the tested films (m²).

2.7. Factorial design

In this paper a Plackett–Burman design was applied for twelve trials in order to evaluate the significance of seven variables grouped as formulation parameters (ratio between water–TEOS, ammonia concentration, ratio between ethanol–water, the presence of an emulsifier hexadecyltrimethylammonium bromide (CTABr)), and as process parameters (agitation rate, temperature and reaction time) upon silica formulation parameters (ratio between water–TEOS, ammonia concentration (mol/L), stirring rate (rpm), temperature (°C), reaction time (h), and CTABr concentration (mol/L)). Each parameter was tested at two levels coded as (−1) for lower level and (+1) for higher level, as it is depicted in Table 1. The STATISTICA statistical package software trial version 10.0 (Stat Soft Inc., Tulsa, USA) was used for experimental design analysis and data processing.

3. Results and discussion

3.1. Microstructure and FT-IR spectra of BC–silica composites

The SEM images of the studied composites were differentiated about the particles size. So, in Fig. 2 are presented samples for which the particles’ sizes were 462 ± 55.75 nm (S1) and 385.4 ± 63.97 nm (S9). Spherical individual particles could be observed entrapped between BC fibrils. SEM pictures for trials S2 and S3 presented in Fig. 3 are rather different. In these cases the particles look like buds on the BC fibrils which are also coated with silica. It is evident that the silica grains are decreasing in size in comparison with samples S1 and S9.

Fig. 4 presents the FT-IR spectra of BC and of composite samples S1, S9, S2, and S7. At first sight one could observe that the spectra of composite materials exhibit both BC and silica absorption peaks and also possible overlapping between some strong bands of the two components: BC and silica. BC spectrum presents characteristic bands like 3341 cm⁻¹ (stretching of hydroxyl groups involved in intramolecular hydrogen bonding), 2984 cm⁻¹ (C–H stretching vibrations), 1645 cm⁻¹ (due to deformation vibrations of the absorbed water molecules), 1160 cm⁻¹ (C–O–C asymmetric stretching vibration from the glycosidic ring), 1106 cm⁻¹ (C–O and C–C stretching vibrations, ring (polysaccharides)) 1054 cm⁻¹ (C–O symmetric stretching of primary alcohol), and 900 cm⁻¹ (CH out of bending vibrations). There are some notable differences between BC spectrum and the spectra of composite materials. Composite spectra present a decrease of the intensity of C–H absorbance group, while the intensity of broad absorbance band in the 3300–3400 cm⁻¹ shifts to higher wavenumber in comparison with BC spectrum. Broaden peak for the BC–silica samples at wavelength 1078 cm⁻¹ could be attributed to an overlapping effect between silica and bacterial cellulose. As it is already known the FT-IR spectrum of colloidal silica particles shows absorption band arising from asymmetric vibration of Si–O at 1090 cm⁻¹ and BC also has characteristic peaks in the same region. For composite spectra it is also visible at peaks 956 cm⁻¹ (S2) and 961 cm⁻¹ (S7) which could be assigned to silica (asymmetric vibration of Si–OH) [24,33–35]. Similar spectra were obtained for cellulose–silica composites and for BC–silica composites [15,24,36].

![Image](image-url)
3.2. X-ray diffraction analysis

Fig. 5 shows XRD patterns for BC and BC–silica composites S1, S2, S5, S9 and S12. Native BC shows three strong Bragg peaks observed at 14.44°, 16.77° and 22.7°. The same peaks are observed for all the studied composite samples which confirm the fact the deposited silica nano and microparticles apparently are not affecting BC crystalline structure. A suggestion of Yano et al. 2008 could be mentioned in relation with our results [26]. The aforementioned authors have obtained BC–silica composite by adding silica sol in the BC fermentation medium and have observed on the XRD patterns that the height of the peak observed at 16.77° decreased as the amount of silica was increased and also that the ratio of the 16.77° peak to the 22.7° peak decreases with the increase of silica content. The explanation of this behavior is the possibility of
silica particles to disrupt the bundling of microfibrils into ribbons. In our experiments silica particles were formed by conducting the Stöber reaction on BC wet membranes in different experimental conditions, so it is also possible that such mechanism of disruption to take place in specific reaction conditions. In Fig. 5 one can observe that there are differences between the intensity of the 16.77° peaks of the BC–silica composites, but we have not found a significant statistical correlation between the intensity of this peak and the silica content of the samples.

3.3. Thermal analysis

The thermal behavior of the composite was studied using thermogravimetric analysis (TG). Typical plots of weight loss versus temperature for the studied samples and BC are presented in Fig. 6. These TG curves indicated a distinct total weight loss between different samples of BC–silica composites. The highest weight loss was obtained for pure BC. Three loss stages were observed for all the studied samples. The first one was between 30 and 200 °C (about 2–6% from the total weight loss) and was due to the water removal or sample dehydration. The second stage which is more significant (about 20–50% from the total weight loss) was between 200 and 400 °C and the third was between 400 and 600 °C, the mass decrease being less than in the second stage (about 10–30% from the total weight loss). The two last stages could be attributed to the thermal degradation and decomposition of BC in the hybrid composites. The residual ash after heating above 600 °C consists of SiO2. Differential thermal analysis (DTA) was also applied for the same samples and the DTA profiles reveal two exothermic peaks over two temperature ranges: 200–400 °C and 400–600 °C. These peaks could be attributed to the decomposition of the organic part of the composites which is the biopolymer (BC). The maximum temperature peaks obtained from DTA are presented in Fig. 7. Our results are similar to those obtained for cellulose silica composites and which were already reported [15,36].

3.4. Water vapor permeability of BC–silica composites

Moisture permeability through a polymer or a composite material is an important characteristic useful in different applications. For example, in some applications, high barrier to migration of gases and vapors is desirable, while for others it is undesirable. The permeability of a material to gases is dependent on a large number of intercorrelated factors. In
measured a WVTR value of 867.203 g/m² day. From the values obtained presents the WVTR values of all composite for composite WVTR could be explained by the model. 0.9234, which indicates that 92.34% of the variability of the response content (%) w/w of the studied samples, these values being presented in

where: Y1 is the mean diameter of the particles (nm) and Y2 is the silica content. For example, Pinto et al. (2008) have measured values of weight percentage of water retention for cellulose–silica composite lower than those for cellulose, the decrease being higher with the increase of silica content [16]. In order to verify whether this assumption is also true for our samples we have tested if a statistical correlation exists between the measured WVTR values and silica content. Because we have not obtained a good correlation, we have tried to see if a statistical correlation is possible between the measured WVTR values, silica content and particle mean diameters measured from SEM images. In this case we have tested a second order polynomial model using STATISTICA software and we have obtained a value for coefficient of determination R² of 0.9234, which indicates that 92.34% of the variability of the response could be explained by the model.

The model equation is:

\[
\text{WVTR} = 248.998 + 0.5853Y1 + 9.329Y2 + 0.00018Y1^2 + 0.03477Y2^2 + 0.01807Y1Y2
\]

(2)

where: Y1 is the mean diameter of the particles (nm) and Y2 is the silica content (%) w/w of the studied samples, these values being presented in Table 2.

even if it is hard to explain the obtained results some remarks could be done. In the Nielsen tortuosity model, which is very popular for permeation, the tortuosity is the main factor which is modified when inorganic substances are embedded in the polymer matrix [39]. Also, the nanoparticulate fillers could produce changes to the polymer matrix itself at the interfacial region and as a consequence influence the barrier properties [40]. We presume that for BC–silica the both mechanisms are present. Hydrogen interactions between silica hydroxyls with OH groups of BC are also possible [25].

3.5. Factorial design results

In Table 1 the factors, codes, low and high levels in Plackett–Burman design were already presented. Analyzing SEM pictures of the samples S1–S12 using Image J Software, we have measured the mean diameter of the particles (Y1) for all the composite films. More than 200 particles were analyzed from SEM images of every sample of BC–silica composites. The results obtained are presented in Table 2 and were used as dependent variable Y1 in the Plackett–Burman design. The second response used in PB design was silica content (Y2) determined as the residual ash after heating above 600 °C and which was considered to consist of SiO2. The TG data for all the samples were used to obtain the percentage of silica in the initial hybrid composites after also taking into account the total weight loss for a sample of silica powder (TG curve for silica powder is not shown). This silica content was used as the second response (Y2) in the PB design. PB design matrix with response values: Y1 (silica particles mean diameter) and Y2 (silica content) are depicted in Table 2.

The analysis of variance (ANOVA) applied to test the availability of PB design for the response Y1 (mean diameter of silica particles) and the results are shown in Table 3. The P-values less than 0.05 indicate that the model terms are significant. Analysis of P-values depicted in Table 3 showed that among the variables tested, ammonia concentration, time and temperature had a significant effect on the silica particle diameter. The model coefficient of determination (R²) has a value of 0.9677, which indicates that 96.77% of the variability of the response could be explained by the model and about 3.23% of the total variation cannot be explained by this model. The F-ratio was found to be far greater than the theoretical value with very low probability (P = 0.007782) for the regression model, indicating that the regression model is significant.

The polynomial model which predicts the silica particles diameter obtained from PB regression analysis is expressed in terms of coded factors as:

\[
Y1 = 212.2201 - 18.8974X1 + 130.0982X2 + 19.4034X3 + 0.744X4 + 41.5857X5 - 61.2249X6 + 63.2426X7 \]

(3)

The Pareto chart could be also used to identify the significant factors. As it could be seen in Fig. 9a three parameters (ammonia concentration (X2), time (X7) and temperature (X6)) have a confidence level greater

![Fig. 8. WVTR values for BC–silica composite samples S1–S12.](image-url)

**Table 2**

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<th>X2</th>
<th>X3</th>
<th>X4</th>
<th>X5</th>
<th>X6</th>
<th>X7</th>
<th>X8</th>
<th>X9</th>
<th>X10</th>
<th>X11</th>
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<th>Y2 (%)</th>
<th>w/w</th>
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![Figure 8](image-url)
than 95% and could be considered as significant. The results are not surprising because it is already known that the concentration of catalyst and temperature are one of the key parameters which influence the particles’ size in Stöber reaction [11,12,27,28]. Arantes et al. have obtained similar results regarding the influence of ammonia concentration upon silica particle size in a factorial experiment for synthesis of colloidal silica nanoparticles [41].

The influence of reaction temperature upon particle size is also well-known in Stöber reaction and there are more explanations for this result. The first one is that if the temperature increases the saturation of ammonia decreases, which then produces a lower dissolution of TEOS and as a consequence a reduced hydrolysis rate which, in turn, decreases particles’ size. The second is that the ammonia concentration could decrease when temperature increases by evaporation. The third explanation sustains that the increase of temperature also increases the nucleation rate and as a consequence the particles’ size is reduced [12,28]. Our results showed that there is a significant influence of temperature, but the minus sign in Eq. (3) suggests that a low temperature is more favorable for the process. This result could be explained by the influence of BC matrix upon the synthesis of silica particles. As it is already stated, the inorganic material is deposited in the biopolymer matrix forming hydrogen bonding between organic and inorganic phases [17,24]. These interactions are powerful enough to influence the particle size and for this reason the increase of temperature is not necessary. But, in the same time, we also agree with the conclusion of Barud et al. [34], who consider that the BC structure influences the size and size dispersion of silica deposed on this biopolymer in a way which is not clear enough.

The second response value which was analyzed was the silica content as it was determined from thermogravimetric analysis. The polynomial model which predicts the silica content of BC–silica composites obtained from PB regression analysis is expressed in terms of coded factors as:

\[
Y_2 = 32.633 - 8.85X_1 - 0.1666X_2 - 1.8333X_3 + 3.0833X_4 + 3.5166X_5 + 6.5166X_6 + 2.6166X_7.
\]

(4)

The analysis of variance (ANOVA) applied to test the availability of PB design for the response Y2 and the results are shown in Table 4.

Analysis of P-values depicted in Table 4 showed that among the variables tested H_{2}O/TEOS ratio, and temperature had a significant effect on the silica content of BC–silica composites. As shown in Table 4 the model coefficient of determination (R^2) has a value of 0.960, which indicates that 96% of the response variability could be explained by the model and the goodness of fit of the model was confirmed. In Fig. 9b the Pareto chart for variable Y2 is also presented which confirms that only two parameters are very significant: water-TEOS ratio and temperature. These parameters are also reported to be very important in silica and cellulose–silica hybrid synthesis [12]. In this case the minus sign for the variable X1 (water/TEOS ratio) suggests that the lower ratio is recommended. It is also known that the ratio between water and TEOS is a crucial factor in Stöber reaction, because the water content influences the rate of TEOS hydrolysis, which in turn affects both the hydrolysis and the condensation reactions in the sol–gel process. For this reason an excess of water is required and should be maintained higher than 2.5 [15]. Because we have worked with higher excess of water (ratio of water/TEOS is in the range from 25 to 100) the low value is enough to ensure the recommended excess water. To enhance the silica deposition on BC membranes our results suggest using a higher temperature, but in order to obtain silica particles with decreasing size we do not need a high temperature. For this reason, in order to optimize the synthesis conditions the response surface methodology will be used in a future work.

3.6. Antimicrobial effect of BC–silica composites

Due to the increasing issues provoked by bacteria adherence and biofilm formation on different medical and industrial surfaces, alternative methods for controlling this behavior are needed [8]. Nanostructured surfaces inhibiting microbial colonization have been developed and optimized in the past 3 years [42]. Even though these modified surfaces proved a great anti-microbial effect, many times they have the disadvantage of being toxic or have a low biocompatibility [43,44].
The use of natural, non-toxic surfaces is the most desired approach in the development of improved surfaces to fight microbial colonization [45]. Our results using BC–silica composites demonstrate that most of the tested surfaces exhibit an anti-adherence and anti-biofilm activity, impairing the biofilm development of *S. aureus*. The most significant inhibitory effect was observed when using S1, S2, S3 and S12 composite samples. These materials proved both anti-adherent and anti-biofilm effects, impairing staphylococcal biofilm development for at least 72 h incubation. On the other hand, the composites S4, S5, S6, S7, S8, S9, S10 and S11 exhibited good anti-adherence profiles, but their inhibitory activity was not maintained over time (Fig. 10). The enhanced efficiency of S1, S2, S3 and S12 composite samples may be due to their better adsorption of the therapeutic agent (usnic acid) and better delivery of it. Yet, additional tests may be needed for revealing the specific action pathway in the bacteria cells since the adhesion of bacteria to mineral surfaces can be affected by a variety of factors including pH, ionic strength and cell surface properties. The first phase of bacterial adhesion and retention is mostly dominated by surface characteristics such as chemical structure, surface hydrophobicity, surface charge, surface free energy, roughness, surface area, pore structure and size [46,47]. Therefore, to better understand the interfacial mechanism, additional knowledge regarding the adhesion forces and mechanisms between bacteria and mineral surfaces is required.

4. Conclusions

BC–silica hybrid materials were prepared using Stöber reaction. A Plackett–Burman design was applied in order to evaluate the significance of process variables upon silica particles size and silica content of BC–silica composites. The analysis of variance (ANOVA) applied to test the availability of PB design has revealed the significant process parameters which influence the silica particles’ size and silica content of BC–silica composites. Silica particles’ size is significantly influenced by ammonia concentration, time and reaction temperature. Silica content is also influenced by reaction temperature and water and TEOS ratio. The BC structure also seems to control the size of silica particles, even if this influence is not very well understood. The SEM pictures reveal that the particles are well dispersed within the BC matrix and for some reaction conditions the silica particles embedded the BC fibrils. Strong interaction between silica and BC was proved by FTIR spectra. From the transport properties, only water vapor transmission rate was measured for the composites. The silica deposited on BC membranes influences this property of the hybrid material, all the BC–silica composites having a lower WVTR value that of pure BC. Our results have demonstrated that it is possible to deposit silica particles on BC wet membranes in the range from 34.5 to 500 nm using different combination between process parameters. This aspect is important in those applications for which the silica particle sizes are playing a key role.

<table>
<thead>
<tr>
<th>Table 4</th>
</tr>
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<tbody>
<tr>
<td>Analysis of variance (ANOVA) for the regression model of silica content using the Plackett–Burman design.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Term</th>
<th>Sum of squares</th>
<th>DF</th>
<th>Mean square</th>
<th>F</th>
<th>P</th>
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<tr>
<td>Model</td>
<td>1225.357</td>
<td>7</td>
<td>175.0510</td>
<td>14.00128</td>
<td>0.011335*</td>
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<tr>
<td>X1</td>
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<td>939.87</td>
<td>52.7745</td>
<td>0.000197*</td>
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<tr>
<td>X2</td>
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<td>1</td>
<td>0.653</td>
<td>0.0523</td>
<td>0.830394</td>
</tr>
<tr>
<td>X3</td>
<td>8.003</td>
<td>1</td>
<td>8.003</td>
<td>0.6401</td>
<td>0.468482</td>
</tr>
<tr>
<td>X4</td>
<td>10.830</td>
<td>1</td>
<td>10.830</td>
<td>0.8662</td>
<td>0.404687</td>
</tr>
<tr>
<td>X5</td>
<td>40.333</td>
<td>1</td>
<td>40.333</td>
<td>2.2260</td>
<td>0.146901</td>
</tr>
<tr>
<td>X6</td>
<td>162.803</td>
<td>1</td>
<td>162.803</td>
<td>13.0217</td>
<td>0.002586*</td>
</tr>
<tr>
<td>X7</td>
<td>23.520</td>
<td>1</td>
<td>23.520</td>
<td>1.8812</td>
<td>0.242099</td>
</tr>
<tr>
<td>Error</td>
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<td>4</td>
<td>12.503</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total SS</td>
<td>1275.36</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P < 0.05 is considered significant.

The BC–silica composites were also tested for their antimicrobial effect, the results obtained demonstrating that some of the samples exhibit inhibitory effect on *S. aureus* biofilm development. These results are encouraging enough to recommend that these composites be used in biomedical applications.

**Acknowledgments**

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**References**


![Fig. 10. Graphic representation of log (CFU/mL) values obtained after the growth of *S. aureus* in the presence of BC–silica–UA composites.](image-url)