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The wettability, mechanical and antimicrobial properties of polylactide/montmorillonite nanocomposite films

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The aim of this study was to evaluate the effect of the not activated (unmodified) montmorillonite (MMT) filler on the antibacterial properties of polymer nanocomposites with a biodegradable polylactide (PLA) matrix. The subject of research was selected to verify the reports on the lack of antibacterial properties of unmodified montmorillonite in nanocomposites and to investigate the potential conditions of their manufacturing which are decisive for the resulting properties.

Evaluation of antibacterial and mechanical properties of both the starting materials and the obtained nanocomposites filled with layered silicates as well as the wettability of the materials, measured by a sitting drop method was made on samples in the form of a film.

The results show that the surface wettability of the polymer nanocomposites did not exhibit significant change compared to the film of neat PLA. However, a significant improvement in the mechanical and antimicrobial properties of the nanocomposite films obtained in a specific solvent casting process of the nanocomposite preceded by exfoliation of the film in an ultrasonic homogenizer was demonstrated. The antibacterial activity against Gram-positive bacteria *Staphylococcus aureus* and *Enterococcus faecalis* was also observed, and, moreover, the montmorillonite-containing films revealed a zone of inhibition of bacterial growth when tested against the lactose-positive bacteria of the *Enterobacteriaceae* family, which are present in the waste water.

The advantageous properties of the obtained PLA/MMT nanocomposites suggest that the unmodified montmorillonite may be potentially used as filler for polymer films in the packaging industry

Key words: *montmorillonite, nanocomposite films, antibacterial properties, mechanical properties*

1. Introduction

Bio-based materials become widely used in packaging due to the increase in environmental concerns over non-biodegradable petrochemical-based plastic packaging materials. Bio-based materials not only include proteins or lipids but also polymers synthesized from bio-based monomers or produced by microorganisms or genetically modified bacteria. Poly (lactic acid) (PLA) is one of them, produced either by the condensation polymerization of lactic acid or by the ring opening polymerization of lactide. PLA is a biodegradable linear aliphatic thermoplastic polyester, and its monomer, which is also the final degradation product, can be obtained by fermentation of carbohydrate feedstocks. PLA has been used not only for disposable plastic products but also in biomedical applications in the form of films, rigid structures, porous scaffolds, and micro or nanospheres [8], [9], [24]. PLA is attractive as a packaging material, due to the tensile strength comparable to that of petroleum-derived thermoplastics, but also due to the ability of degradation under commercial composting conditions. Furthermore, PLA is resistant to oil and has a good water vapour permeability in combination with a better oxygen barrier property compared to all other bioplastics [11], [17], [19], [23]. However, the gas barrier properties of pure PLA, its low resistance to solvents (e.g., against water) and other properties such as low

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thermal stability and brittleness, are often insufficient for food packaging applications [1]. Thus, direct polymer blending, multilayer films and polymer nanocomposites (PNCs) aimed at solving the aforementioned problems, which cannot otherwise be attained with a neat polymer material. Hence the multilayer films and polymer blending possess higher production and material costs and other drawbacks associated with recycling, the use of fillers in nanocomposites has attained a broad attention aiming at improving the properties of bio-based packaging materials. Filler materials can include clay and silicate nanoparticles, silica nanoparticles [13], [21], [25], carbon nanotubes [22], graphene [6], nanostarch [8], cellulose nanofibers or nanowhiskers [14], chitin or chitosan nanoparticles [20] and others. Among others, the use of nanoclay fillers, particulary montmorillonite, has gained considerable attention and resulted in significant improvements in mechanical, gas barrier, and optical properties at low filler content (less than 5% of weight) [21]. Numerous studies have been performed on the PLA-based nanocomposites to exploit the enhanced properties of such nanocomposites, as already mentioned [4], [8], [14], [23].

Great attention was also given to the antimicrobial properties of PLA-based composites, as one of the most important features, which qualifies the material to be accepted as a packaging material in contact with food. PLA alone shows antibacterial activity when used in solution of oligomers or in combination with certain organic acids or antibacterial agents [2], [3], [15]. There have also been many studies on the antibacterial activity of PLA composites containing various fillers such as nano cellulose, pectin particles, but above all nanoclays [11], [14], [21]. It was reported by Rhim et al. in 2006 [20], that some nanocomposite films prepared with an addition of a certain organically modified nanoclay had the strong antimicrobial activity against both Gram-positive and Gram-negative bacteria. It was postulated that the antimicrobial functionality of the nanocomposite films could be attributed to the quaternary ammonium groups of organically modified clays. In more recent research Rhim et al. in 2009 [21] found that among the PLA-based composite films with different types of nanoclays, such as Cloisite Na^+, Cloisite 30B and Cloisite 20A, which were prepared using a solvent casting method, only PLA/Cloisite 30B (MMT modified with a quaternary ammonium ion containing methyl tallow bis-2-hydroxyethyl) composite film showed a bacteriostatic activity against *Listeria monocytogenes*. Similar findings on the antibacterial activity of montmorillonites were reported by Liu et al. in 2014 [10] in a study on polyvinyl alcohols-based (PVOH) nanocomposite films filled with various types of montmorillonite nanoclays, including organically modified MMT clays and a natural MMT (Na^+-MMT), fabricated by the solution-intercalation. It was noticed, that only the addition of octadecyl ammonium modified clay into PVOH films showed remarkable antimicrobial activity against Gram-positive bacteria *L. monocytogenes* and *S. aureus* (natural Na^+-MMT showed no antibacterial activity). Also, Darie et al. in 2014 [7] studied the effect of nanoclay hydrophilicity on the properties of PLA/clay materials showing that nanocomposites filled with Cloisite 93A (montmorillonites organically modified with methyl dehydrogenated-tallow quaternary ammonium) revealed antibacterial properties against both Gram-positive and Gram-negative bacteria. There has been a lot of paper also published on the antimicrobial activity of PLA nanocomposites with clay fillers intercalated simply by antibiotics and/or other bacteria killing substances, however, the biocidal effect was rather explainable and could be attributed rather to the antimicrobial agents used as intercalants than to the materials used as excipients modifying the kinetics of antimicrobials release [12]. It should be also mentioned that since the antimicrobial properties have been reported, among others, for metal ions such as Ag^+, Cu^{2+} and Ni^{2+} and quaternary ammonium compounds, a good antibacterial activity observed in the case of the already mentioned organically modified clays, i.e., Cloisite 30A can be relatively easy explained. Furthermore, Busolo et al have published the biocidal effect revealed by the biocomposite films based on silver containing melt-compounded polylactide-clay materials [5]. In that study, the organoclays with native silver nanoparticles and silver in a ionic form showed strong antimicrobial activity against *S. aureus*.

Although various reports were published on the antimicrobial properties of PLA-based nanocomposites filled with both modified (organophylized) and intercalated clays, only a few of these works related to antibacterial properties of PLA films filled with unmodified montmorillonite. The primary objective of this study was to obtain the composite films PLA/unmodified montmorillonite (PLA/MMT) and to discuss their (unprecedented) good antimicrobial properties in the context of previously reported results, as well as to evaluate the effect of concentration of filler on the antibacterial activity of these materials in terms of their suitability for food packaging. The clay filled nanocomposite films were manufactured using a specific solvent casting process preceded by exfoliation of the filler in an ultrasonic homogenizer and a significant improvement in mechanical and antimicrobial properties was demonstrated.

2. Materials and methods

2.1. Materials

Poly-l-lactide (Ingeo, PLA 3051D) manufactured by Nature-Works, USA, and montmorillonite (MMT) Veegum®F (R.T. Vanderbilt Company, INC.) were used in the study. Dichloromethane purchased from Sigma-Aldrich, Poland and 70% 2-propanol from AVANTOR, Poland were also used as additional chemicals. The nutrient broth was also used (formula in accordance with the quality certificate of BIOCORP, Poland) to feed *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 19433, while peptone water with lactose (BTL, Poland) was used for *Escherichia coli* ATCC 25922 and bacterial suspension of the active lactose-positive sludge from a chamber of water treatment plant for the *Enterobacteriaceae* family.

2.2. Preparation of PLA/MMT films

Both PLA and PLA/clay nanocomposite films (PLA/MMT) were prepared from the polymer solution by solvent casting. In the first stage, a lactic acid polymer (PLA) in the form of pellets was dissolved in dichloromethane (DCM, Sigma-Aldrich) in a weight ratio of 1:10 and the solution was stirred using a magnetic stirrer (ES type manual Wigo 24) for 6 hours to ensure good dissolution and homogenization. Reference samples of neat PLA (with 0% nanofiller) were directly cast in the form of film (2 g of PLA per sample) in Petri dishes. Nanofillers used to produce the individual nanocomposite samples were weighed using an analytical balance and then added to 5 ml of dichloromethane per each sample, and mixed for 2 minutes using an ultrasonic homogenizer (Vibra Cell VCX 130, Sonics company) to break up agglomerates and to increase the dispersion of the nanoadditive in the slurry prior to mixing with the solution of PLA. Then the individual suspensions of nanofiller were added to 20 ml of the polymer solution (2 g of PLA sample) and mixed, and the resulting suspension was again introduced to the ultrasound homogenizer. The suspensions after homogenization were then poured onto separate Petri dishes, which were left for 24 hours in a fume hood at room temperature to evaporate the solvent, however, in order to ensure complete evaporation, the samples were placed in a vacuum oven SPT-200.

Twenty samples in the form of a film, with a diameter of 10 cm, were made out of each suspension as well as twenty (film) samples of pure PLA were additionally prepared as a reference (control) material, and the compositions of the nanocomposites obtained are shown in Table 1.

Table 1. The compositions of the obtained nanocomposite film samples

Material/Film	Content of the nanofiller in 2 g of PLA		
	% vol.	% wt.	weight [g]
PLA/MMT	1	2.5	0.051
	1.5	3.8	0.076
	2	5.0	0.100

2.3. Methods

The following tests were carried out on all the composite materials in the form of a film:

- contact angle test by a sessile drop method using a goniometer DSA10Mk2 Kruss, 10 repetitions of the test were made for each of the nanocomposite,
- test of mechanical properties using a Zwick testing machine 1435-PUG; representative segments of the films with a width of approx. 5 mm and a thickness of approx. 0.18 mm were used as the samples (testing speed 2 mm/min); tensile strength, elongation at break and modulus of elasticity were evaluated based on measurement according to the ISO 527-3 standard; testing of mechanical properties was performed on 20 samples of each material,
- antimicrobial activity of the starting materials (powdered clay materials and film of a neat PLA) as well as of the nanocomposites in the form of a film; measurements were performed on the following reference strains granules: *Staphylococcus aureus* ATCC 25923 (Gram-positive granuloma), *Enterococcus faecalis* ATCC 19433 (Gram-positive granuloma), *Escherichia coli* ATCC 25922 (Gram-negative bacillus) and a sample of sewage bacteria from the chamber of sludge – lactose-positive bacteria of the *Enterobacteriaceae* family was isolated.

The antibacterial properties of the powdered (starting) montmorillonite filler were investigated using samples of 0.05 g, 0.025 g, 0.013 g, 0.006 g, respectively, which were weighed in sterile test tubes (max. concentration 5%). The samples were disinfected at a temperature of 160 °C for 1 hour prior the proper inoculation, and then, 1 ml of liquid nutrients was added: nutrient broth (composition according to the certificate of quality Biocorp and BTL) for *Stapylococcus aureus* and *Enterococcus faecalis* strains, peptone water with lactose (BTL, Poland) for *Escherichia coli* and the bacteria suspension of lactose-positive bacteria isolated from the sample of raw sewage (*Enterobacteriaceae* family), accordingly. A mixture of 2 ml of bacterial suspension with the Ringer's solution was prepared for inoculation, which corresponds to 0.5 degree in the McFarland scale, i.e., approximately 1 to $2 \cdot 10^8$ CFU (colony forming units). The absorbance was measured using a Merck Pharo 100 photometer and the measured absorbance of suspension at a wavelength of 600 nm ranged from 0.08 to 0.1 for the 0.5 degree in the McFarland scale. Each tube with the powdered montmorillonite was inoculated with the 10 μl inoculum prepared from bacterial suspension (where the expected number of CFU in the tube was 10^6), and then the tubes were moved to an incubator and incubated at 37 °C for 24 hours. The presence of bacteria was visualized by a color change of the medium from red to yellow (pH indicator) during a test for *Escherichia coli* and a suspension of lactose-positive bacteria isolated from samples of raw sewage (family *Enterobacteriaceae*). In other cases, additional test for the presence of bacteria was conducted after incubation, i.e., inoculation was made on a permanent TSA substrate with the use of a scratch tubing and then samples were incubated for 24 hours in an incubator at 37 °C.

The antibacterial properties of the nanocomposite films were investigated on samples having a diameter of 6 mm, but due to the nature of the polymeric matrix disinfection could not be carried out at elevated temperature. Therefore, it was completed by the use of the disc-diffusion method according to the following procedure: a sterile Petri dish was rinsed with an atomizer containing disinfectant agent (70% 2-propanol, AVANTOR, Poland), then sterile forceps were used to put the discs on the wet bottom of the dish and the samples were sprayed with disinfectant agent, and then the dish was closed and the disinfection agent was allowed to evaporate at ambient temperature.

The antibacterial tests on foils started with the proliferation of certified reference strains obtained in pellets and the same nutrient substances were used to feed the bacterial cultures, as in the case of test on powdered montmorillonite, i.e., nutrient broth (formula in accordance with the quality certificate of BIOCORP, Poland) for *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 19433, and peptone water with lactose (BTL, Poland) for *Escherichia coli* ATCC 25922, and bacterial suspension of the active lactose-positive sludge from a chamber of water treatment plant for the *Enterobacteriaceae* family. The sterile forceps were used to transport the pellets, which were dissolved in 2 ml of Ringer solution in a Eppendorf tube, and then left to stand still for 15 minutes. A sterile flask was filled with 10 ml of appropriate liquid nutrient, maintaining the principle of sterility, and 100 μl of the prepared bacterial suspension (with reference strains) or 100 μl of sewage sample, was then inoculated to the flask with nutrient. The flask with appropriate description was further placed in an incubator at 37 ± 2 °C (or 34 ± 1 °C for *Staphylococcus aureus*), and the incubation was carried out from 18 to 24 hours.

Before the proper inoculation of the nanocomposite samples, the Petri dishes with TSA agar (Tryptic Soy Agar) were prepared and 40 g of a substrate was immersed in 1000 ml of distilled water and sterilized at 121 °C for 15 minutes. Agar was then carefully mixed, and poured on plates of 90 mm diameter (15–20 ml) and left until drying. Successively, a suspension of 0.5 in the McFarland scale was prepared, in the same manner as for the powdered samples, and the inoculation was initiated 15 minutes after the preparation of suspension, and for this purpose, a sterile swab was dipped in suspension and the excess was blotted on the container walls. Inoculation was made at three locations on the prepared dish with the solidified TSA agar, each time turning the dish by 60°. When the surface of agar solidified, the sterile forceps were used to load the sample disc film on the agar surface lightly pressing to the substrate. The plates were moved to an incubator and incubated for 18 to 24 hours at 37 ± 2 °C, and the inhibition zone was measured after the incubation time using a caliper (including the diameter of the disc).

The test results of antimicrobial activity for the PLA/MMT nanocomposites in the form of a film are the average of 3 measurements made for each type of material.

3. Results

3.1. Contact angle and wettability

The test results of the contact angle showed that the introduction of montmorillonite in the form of nanofiller (in the range of 0.5–2.0% vol.) into the PLA matrix does not change significantly the surface character of the nanocomposite compared to a pure unmodified PLA (Fig. 1).

Contact angle was measured on both sides of the obtained samples (↑ denotes the upper side of nanocomposite, ↓ denotes the bottom side of nanocomposite from the side of a dish), and the results were averaged at both sides of films.

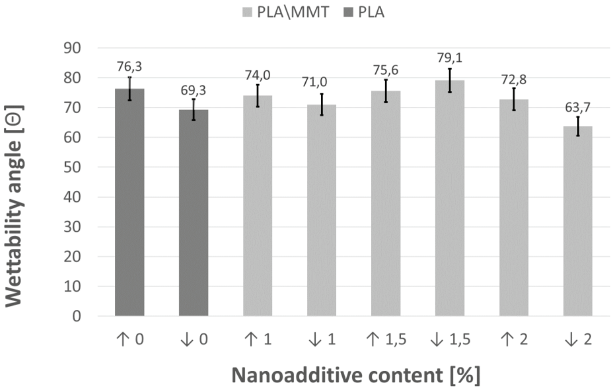

Fig. 1. The contact angle results depending on the type and content of the nanoadditives, as well as on the side of a sample (↑ and ↓ denote the top and the bottom of sample respectively)

3.2. Mechanical properties

The detailed test results of mechanical properties of both PLA and PLA/MMT (film) materials are shown in Table 2, where the values of tensile strength, modulus of elasticity and elongation at maximum load are shown respectively (the standard deviation was also evaluated for each material and test series).

The tests were conducted on the representative strips of the films with a width of approx. 5 mm and a thickness of approx. 0.18 mm. The test was carried out on 20 samples of each material at test speed of 2 mm/min, and the typical stress–strain curves for all the tested materials are shown in Fig. 2.

Table 2. Mechanical properties of PLA and PLA/MMT nanocomposite films

Material/Film	Tensile strength [MPa]	Elastic modulus [GPa]	Strain at Load$_{max}$ [%]
PLA	21.58 ± 3.60	1.14 ± 0.30	3.92 ± 0.89
PLA/MMT 1% vol.	40.11 ± 2.82	2.35 ± 0.18	2.13 ± 0.09
PLA/MMT 1.5% vol.	52.63 ± 4.74	2.95 ± 0.37	2.92 ± 0.47
PLA/MMT 2% vol.	30.56 ± 2.57	2.01 ± 0.24	2.20 ± 0.27

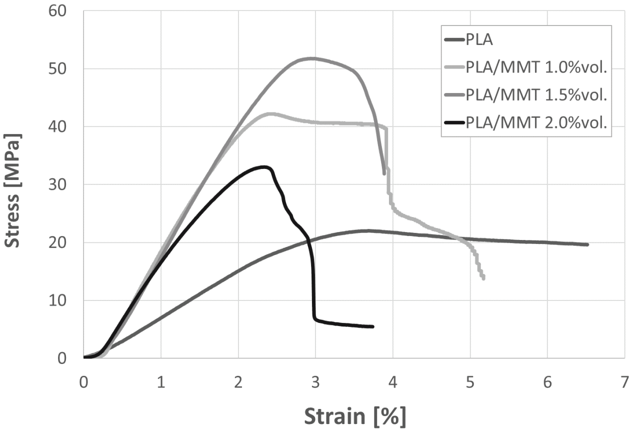

Fig. 2. Typical stress–strain curves for neat PLA and PLA/MMT nanocomposite films

Table 3. The antibacterial effect of the starting MMT powders at different concentrations of clay

<table>
<tr><th>Model bacteria strain</th><th colspan="5">Type and concentration of substrates [g/ml] /
presence of microorganisms in sample after 24 h incubation</th></tr>
<tr><td rowspan="5"><i>Staphylococcus aureus</i>
ATCC 25923</td><td rowspan="4">MMT</td><td>0.0500</td><td>+</td><td rowspan="5"><i>S. aureu</i> control</td><td rowspan="5">+</td></tr>
<tr><td>0.0250</td><td>+</td></tr>
<tr><td>0.0125</td><td>+</td></tr>
<tr><td>0.0063</td><td>+</td></tr>
<tr><td>control</td><td>0.0063</td><td>–</td></tr>
<tr><td rowspan="5"><i>Enterococcus faecalis</i>
ATCC 19433</td><td rowspan="4">MMT</td><td>0.0500</td><td>+</td><td rowspan="5"><i>E. faecalis</i> control</td><td rowspan="5">+</td></tr>
<tr><td>0.0250</td><td>+</td></tr>
<tr><td>0.0125</td><td>+</td></tr>
<tr><td>0.0063</td><td>+</td></tr>
<tr><td>control</td><td>0.0063</td><td>–</td></tr>
<tr><td rowspan="5"><i>Escherichia coli</i>
ATCC 25922</td><td rowspan="4">MMT</td><td>0.0500</td><td>+</td><td rowspan="5"><i>E. coli</i> control</td><td rowspan="5">+</td></tr>
<tr><td>0.0250</td><td>+</td></tr>
<tr><td>0.0125</td><td>+</td></tr>
<tr><td>0.0063</td><td>+</td></tr>
<tr><td>control</td><td>0.0063</td><td>–</td></tr>
<tr><td rowspan="5">Lactose-positive bacteria
of the <i>Enterobacteriaceae</i> family,
isolated from the sewage</td><td rowspan="4">MMT</td><td>0.0500</td><td>+</td><td rowspan="5">lac+ control</td><td rowspan="5">+</td></tr>
<tr><td>0.0250</td><td>+</td></tr>
<tr><td>0.0125</td><td>+</td></tr>
<tr><td>0.0063</td><td>+</td></tr>
<tr><td>control</td><td>0.0063</td><td>–</td></tr>
</table>

Table 4. The result of the assessment of bactericidal effect for PLA and PLA/MMT films

Model bacterial strain	Diameter of inhibition zone [mm]			
	PLA/MMT composites (%vol. fraction of MMT)			Neat PLA
	1	1.5	2	
S. aureus ATCC 25923, absorb. 600 nm: 0.089	7	10	10	0
E. faecalis ATCC 19433, absorb. 600 nm: 0.100	7	9	8	0
E. coli ATCC 25922, absorb. 600 nm: 0.098	0	0	0	0
Lactose-positive bacteria of the *Enterobacteriaceae* family, absorb. 600nm: 0.100	9	10	14	0

3.3. Antibacterial properties

The test results of antimicrobial activity for the starting MMT powders are summarized in Table 3. The test was carried out with different concentration of MMT in the subsequent suspensions, expressed as the amount of MMT in grams per milliliter of suspension. The signs (+) and (–) denote the presence of bacteria in the sample after incubation time. There were also two types of control samples used: the first for MMT material alone (without bacteria) and the second for the subsequent bacteria (without MMT substrate) and it could be seen that those controls worked properly, which indicated that the tests were performed properly.

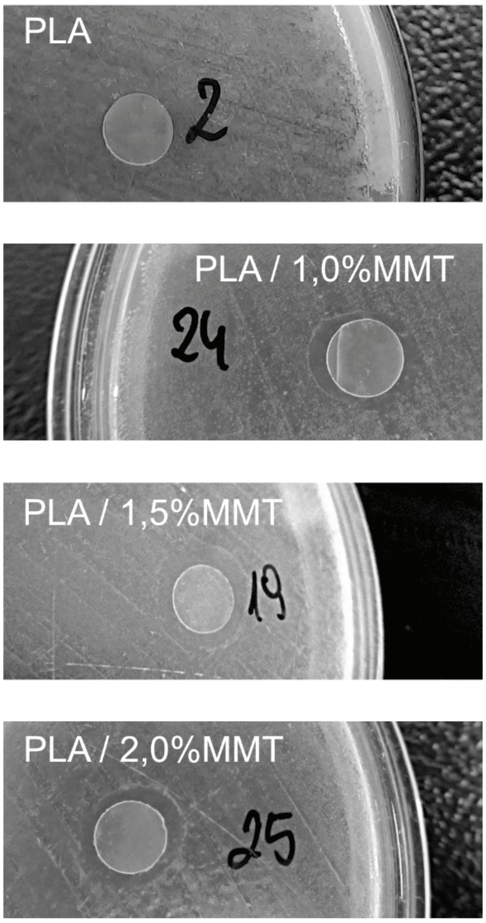

Fig. 3. Exemplary inhibition zones of *Staphylococcus aureus* growth after 24 hours of incubation of PLA and PLA/MMT films

The detailed results of bactericidal effect for both PLA and PLA/MMT nanocomposites in the form of a film are summarized in Table 4. A bactericidal effect for nanocomposite foils was tested on the samples in the form of discs with a diameter of 6 mm and the bactericidal activity was evaluated based on the inhibition zones of bacterial growth, which appeared around the discs.

Exemplary photos of inhibition zones of *Staphylococcus aureus* growth, appearing around the samples after 24 hours of incubation for PLA/MMT films and the reference sample, which was a disc of neat PLA, are shown in Fig. 3.

4. Discussion

4.1. Characterisation of PLA and PLA/MMT films

The values of contact angle for all the nanocomposite samples ranged from 60 to 80 degrees, whereas the contact angle for a neat PLA was 72.8 degree, which confirms rather good wettability of all the tested materials. The lowest wettability was observed for the sample with the highest volume fraction of nanofiller (2%), which may eventually lead to the conclusion that a higher content of clay may affect the surface properties of nanocomposites, which in turn may affect numerous properties such as the ability to degrade as well as the degradation rate. The accelerating influence of clay filler content on the degradation rate of PLA/MMT composites has been previously reported [18]. On the other hand, Liu et al. [11] suggested that, due to the hydrophobic nature of PLA, microorganisms cannot easily access the film, thus in consequence, no antimicrobial activity was observed in the PLA/clay composite films. In this study, the measured contact angles suggest that both neat PLA and PLA/MMT composites are generally not inherently hydrophobic (contact angles well below 90 degree), and the partial swelling is possible in this system. It may trigger a different kind of interaction between microorganisms, humid environment, in which they live and polymeric or nanocomposite foils. The most interesting, however, is that even though there are no significant differences in the wettability between neat PLA and PLA/MMT composite foils, the antimicrobial activity of these materials is fundamentally different, and the observed differences will be described in detail in the following sections. Anyway, the differences in antimicrobial activity cannot be explained simply by the changes in wettability.

It can be clearly seen from the results of mechanical tests, that the introduction of montmorillonite into the polymer matrix increased strength and tensile modulus of nanocomposites filled with a relatively small volume fraction of filler. The average strength of the unmodified film of polylactide (PLA) was measured as 21.58 MPa, while the obtained nanocomposites with 1% vol. of nanoadditive were characterized by a twofold increase in tensile strength compared to a film of pure PLA. In turn, the PLA/MMT nanocomposites containing 1.5% vol. of clay revealed the highest strength of 52.63 MPa, which was nearly 2.5 times higher as compared to films of pure PLA. A significant decrease in tensile strength was then recorded for nanocomposites with 2 %vol. of nanoadditive in relation to nanocomposites filled with 1.0 to 1.5% vol. of MMT, however, the measured strength was still higher than for the samples of PLA not filled. For all the tested samples a relatively small error indicated a good dispersion of nanoadditives in the polymer matrix.

The results indicated that the addition of montmorillonite increased the tensile strength of the polymer matrix, however, above 2% vol. (which is a 5 wt % of montmorillonite), a decrease in the strength of the nanocomposite films was observed. Too much nanoadditive or did not positively affect or even reduced the mechanical properties of the PLA/MMT nanocomposites [21].

A similar trend can be seen also in the results of the modulus of elasticity (E) for which, it was visible that the average modulus of the films of pure unmodified PLA was 1.14 ± 0.30 GPa. and the observed increase in modulus value was 2 times and 2.5 times for nanocomposites filled with 1% vol. and 1.5% vol. of MMT respectively. The maximum value of the modulus 2.95 ± 0.67 GPa was reported for 1.5% vol. of clay in the nanocomposite, and a substantial decrease in the value of modulus was again observed for 2% vol. of clay addition.

The extraordinary improvement in the mechanical properties provided by the unmodified clays was previously reported, among others, by Park et al. [16] who compared the effect of clay modification on tensile properties of polymeric nanocomposites using unmodified montmorillonite (Cloisite Na+) and modified montmorillonite (Cloisite 30B) as fillers. Surprisingly, that study revealed that the polymeric composites filled with unmodified montmorillonite showed improvement over the composites filled with modified (activated/organophylised) montmorillonite. The improvement observed in unmodified montmorillonite nanocomposites can be explained by the higher hydrophilicity of unmodified montmorillonite compared to modified montmorillonite. The polymeric composites used in that work were highly hydrophilic what led to the intercalation along with better dispersion with hydrophilic unmodified montmorillonite than organophilic modified montmorillonite.

It is also worth noting that the elongation at break (and the elongation at maximum load) decreased upon introduction of MMT into the polymer matrix, which is typical for polymer composites in which the addition of a filler usually leads to a reduction in elongation and reduction of creep phenomenon due to the increased rigidity of the polymer bonds influenced by the presence of filler. However, an interesting and in some ways rather unusual is the increase of elongation at maximum load up to 2.9% in the case of PLA/MMT nanocomposite film with 1.5% vol. addition of montmorillonite (compared to 1% and 2% vol. of the filler). On the other hand, this increase can be explained rather easily by the significant increase in tensile strength for this material, resulting also in a proportional increase in the elongation at break.

4.2. Antimicrobial activity of PLA and PLA/MMT films

As previously mentioned, Liu et al. [11] in their research concluded that due to the hydrophobic nature of the PLA the microorganisms cannot readily access the film and, therefore, antibacterial activity of the film/clay composite PLA should rather not be expected. However, as also mentioned in the section on the results of the contact angle, the fundamental differences in antimicrobial activity were found between the pure PLA and PLA/MMT composite film, although there was no significant difference in their wettability and the corresponding contact angles were well below than 90 degrees.

The results of the study on antibacterial activity indicated that the starting MMT clay alone (powdered MMT of various concentrations) used as a filler in the PLA/MMT nanocomposites showed no bactericidal properties. The powders were tested for bactericidal activity against *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli* and bacteria sludge from the *Enterobacteriaceae* family, and it was found, after 24 hours of incubation, that in all the samples the bacteria were present. During the tests against *Escherichia coli* and a suspension of lactose-positive bacteria isolated from a sample of raw sewage (*Enterobacteriaceae* family), the presence of bacteria was monitored by the color change of nutrients from red to yellow (pH indicator), in other cases, discoloration and cloudiness of nutrient were observed. In order to confirm the presence of bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*, and the bacteria sludge from the *Enterobacteriaceae* family) in suspensions of powders, bacteria were seeded onto the TSA agar by a scratch tubing. The results were confirmed during re-reading after 24 h of incubation at a temperature suitable for growth of the individual bacteria. The evaluation of bactericidal effect of the starting materials in the form of powder of various concentrations was carried out using the same procedure, which was described in the method chapter.

Although the starting clay showed no bactericidal properties in relation to any of the bacterial strains tested, nanocomposites of a film containing montmorillonite showed bactericidal activity against *Staphylococcus aureus*, *Enterococcus faecalis*, and the bacteria from the *Enterobacteriaceae* family obtained from sewege sludge. In the case of evaluation of bactericidal effect against *Escherichia coli*, for any composite foil, as in the case of montmorillonite alone, there was no zone of inhibition, which might indicate a lack of antibacterial activity of the obtained MMT-based films against this bacteria.

Analyzing the above results, it can be stated that the obtained nanocomposite films, containing montmorillonite dispersed in a polylactide, exhibited pretty unexpected antibacterial properties. Previously, the extraordinary antimicrobial activity was reported rather for the surface activated montmorillonite, but in our study montmorillonite has not been surface-modified/activated prior to introduction into the polymer matrix. Thus, the bactericidal effect can be triggered not only by the activation, where the antibacterial activity is rather provided by the nature of the activating agents which are often poisonous or toxic, but also by a method of preparation of fillers and composites. The starting powders as well as the nanocomposite films prior to casting were thoroughly homogenized with the use of ultrasonic mixing and, as a result, the samples exhibited a very good homogeneity, as evidenced by the repetitive results of the tests of physical properties carried out in this work.

All steps of ultrasonic mixing performed have also led to a very substantial increase in the interlayer distance of montmorillonite or even complete exfoliation of the clay in a volume of polymer matrix, as evidenced by the tests carried out by the small-angle X-ray scattering (SAXS), in which there were no peaks on the intensity curve for the tested nanocomposites.

5. Conclusions

The aim of the study was to obtain polymer nanocomposites with the unmodified montmorillonite filler as well as to investigate their bactericidal and mechanical properties. MMT was used as a filler and a biodegradable polylactic acid polymer as a matrix to produce nanocomposites in the form of a film with significantly improved mechanical properties and a barely noticeable change in wettability, compared with a neat polylactide film.

It was found that the introduction of montmorillonite (MMT) into the polymer matrix caused no apparent change in the nature of the surface properties of nanocomposite compared to the neat, unmodified PLA, however, the addition of clay contributed to a great increase in mechanical properties of the nanocomposites. The highest strength was tested for the nanocomposites with 1.5% vol. fraction of montmorillonite filler.

The evaluation of antibacterial activity of both the starting materials and the resulting nanocomposites was also carried out, which has led to surprising and unexpected findings, since the bactericidal effect was reported for the obtained nanocomposites. Finally, it was demonstrated that montmorillonite when well dispersed or even exfoliated in the polylactide matrix, is able to trigger the bactericidal activity against Gram-positive bacteria *Staphylococcus aureus* and *Enterococcus faecalis*, as well as against a lactose-

-positive bacteria from *Enterobacteriaceae* family present in the wastewater, but does not affect the antibacterial activity against Gram-negative bacteria.

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