Evaluation of the possibility to use wood-polymer composites for treatment of wastewater from the septic tank

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ABSTRACT

In this study, the susceptibility of the surface of fittings prepared using wood-polymer composites to the formation of biofilm in the process of wastewater treatment in a model laboratory system was investigated. The conducted analyses indicate the beneficial effect of the tested solid substrate on the long-term settlement of various microorganisms, especially in case of supports prepared using wood-polymer composites. The conducted studies allowed to establish that the initial hydrophilicity of the material, which determines its high wettability, is the factor that contributes to the formation of biofilm on fittings made of a wood-polymer composite with a composition of 60% wt. of polyethylene Tipelin polypropylene and 40% wt. of C120 wood flour filler. Microorganisms firmly anchored to a solid substrate form a biofilm and trigger numerous reactions in the surrounding environment. The process of wastewater treatment on the carriers was effective due to the presence of numerous microorganisms. The formation of a biofilm on the surface of the composites ultimately resulted in an increase of the contact angle, which was confirmed by repeated studies performed after the end of the tests.

Keywords: Biological beds; Moving bed biofilm reactor; Wood-polymer composites; Wastewater treatment technologies; Wettability

1. Introduction

In some cases, treated or insufficiently treated wastewater discharged into running or standing waters or into the ground poses a serious threat to the natural environment. In terms of economical regards, efficient wastewater treatment has a significant impact on saving water and prevents its unnecessary losses. Reuse of treated and pre-treated wastewater (e.g., for irrigation of green areas) requires the use of appropriate methods and wastewater treatment systems. Biological contaminants present in wastewater are very diverse and are represented, inter alia, by various bacteria (e.g., coliform bacteria, Escherichia coli, Salmonella spp., Shigella spp., Vibrio cholerae), parasite cysts and eggs, protists, viruses and fungi. Depending on their type and quantity, all these forms can be dangerous to both the environment and human health [1,2]. The effectiveness of wastewater treatment, the flexibility of the used method and its resistance to chemicals contained in the wastewater flowing into the device largely depend on the technology used. Currently, wastewater is most often treated biologically after
mechanical treatment. The primary contaminants which must be removed from wastewater are carbon, nitrogen, and phosphorus, including organic compounds, ammonia, phosphates, and many other pollutants. These processes involve the oxidation, transformation and removal of dissolved pollutants present in the wastewater with the participation of microorganisms. Microorganisms involved in these processes can be attached to the substrate, creating the so-called biological membrane or be suspended in the form of the so-called activated sludge. Biological processes are carried out for example in biological beds. Biological beds can be divided into fixed beds, which include flooded beds, drip and submerged beds, as well as moving beds, which include disc and suspended beds [3–5]. Based on their definition, biological beds are technical devices that use the natural phenomenon of proliferation of microorganisms on a given substrate (filling) during the decomposition of organic compounds. There are three basic stages during the formation of biofilm: settlement (attachment of microorganisms to the substrate due to van der Waals and electrostatic forces), colonization and growth, that is, maturation of the biofilm. Biofilms consisting of numerous microorganisms prefer substrates with increased porosity. The processes of biological decomposition carried out by settled microorganisms in the outer part occur under aerobic conditions (aerobic layer), an anaerobic layer is formed while in the inner part, in which some substances are decomposed in anaerobic processes [3,5–7]. Currently, the biological membrane carriers used in the moving bed technology are made of natural or artificial materials. The fillers from synthetic materials consists of various types of loose fittings or packages (blocks) made of polyvinyl chloride, from synthetic materials consists of various types of loose biological membrane carriers used in the moving bed technology are made of natural or artificial materials. The fillers from synthetic materials consists of various types of loose fittings or packages (blocks) made of polyvinyl chloride, polyamide, polypropylene or polyethylene. Depending on the type of material, its shape and filler size, they have a specific surface area in the range of 240–2,000 m²/m³ [8]. The treatment efficiency depends on the supplied pollutant load – the bed load. The type of filling and the aeration rate also influence the treatment efficiency. The possibility of using a given carrier in a biological deposit is also influenced by its building material as well as its affinity for the adsorbed biomass [3,9]. Compared to other fixed biomass systems (trickling filters and submerged biofilters), the MBBR (moving bed biofilm reactor) does not experience clogging problems and has lower pressure losses [10,11].

The qualitative and quantitative composition of the organisms that form the biofilm plays a very important role in the process of biological wastewater treatment. According to different authors, the dominant role in these systems is played by bacteria, ciliates and rotifers as well as amebas, flagellates and nematodes, which are adapted to living both in aerobic and anaerobic conditions [1,2,7,12–14].

The use of polymer composites with natural fillers (mainly wood-polymer composites, WPC) as carriers in the biological bed technology is an interesting and new solution, mainly due to the large active surface available for the development of a biological membrane. There are many definitions which describe wood-polymer composites, but the most precise one is considered to be the one representing WPC as thermoplastically processable composites consisting of wood, polymer material and additives [14–19]. The dynamic development of the production of wood-polymer composites results, among others, from their good performance properties due to an advantageous combination of the features of the polymer components and the natural additive. An additional role is played by ecological considerations, because it is possible to use incomplete and post-consumer wood for the production of WPC, which is classified as waste. Another important factor is the possibility to recycle the worn out composite materials and, ultimately, use them to recover energy via combustion [20–28].

The economic factor, that is, the increasing prices of raw materials for the production of polymers (oil and gas), as well as the very high demand for these materials, result in the application of polymer matrix composites in an increasing number of industries, including in the wastewater treatment technologies. In addition, the physical properties of WPC composites, which depend, for example, on the type and amount of the filler and the method of its distribution in the matrix, as well as on the orientation of the fibres in case of fibrous fillers, affect the adsorption and aggregation of microorganisms on the surface of the composite. Therefore, the phenomenon of wetting the surface of the bed material by other substances or biological matter is an important aspect which should be taken into account when analysing the processes that occurs on the surface of a moving biological bed. Wettability (determined by the contact angle) is also related to the free surface energy, which determines, among others, the rate and efficiency of microbial aggregation, as well as the hydrophilicity or hydrophobicity of carriers [29–31].

The studies of various authors conducted to date indicate the possibility of using pure PE (polyethylene), PVC (polivinyl chloride), PP (polypropylene), ABS (acrylonitrile-butadiene-styrene terpolymer), PS (polystyrene), PC (polycarbonate), or polyester-glass laminates as biofilm carriers [3,5,7,10,32–37].

The study presents the results of research work aimed at determining the possibility of using wood-polymer composites as a material to create elements of a biological bed. The conducted research included the characterization of the population of microorganisms living on composite supports and PE supports (reference material) and the assessment of the contact angle of the material surface, as it most likely affects the microbial aggregation.

2. Materials and methods

The research material was a wood-polymer composite (WPC) and a comparative pristine polyethylene. Polyethylene (Tipelin BA550-13) was used as the matrix of the composites, while wood flour from conifers: Lignocel C 120 with particle size 70–150 µm (J. Rettenmaier and Söhne GmbH CoKG) was the filler.

Homogenization of polymers with wood flour was carried out in the single-screw extrusion process; a composite granulate was thus obtained. The temperature of the extruder heating zones ranged from 120°C to 160°C, the rotational speed of the extruder screw was 50 rpm. A simple cylindrical support with a corrugated surface was produced by extrusion from a composite material which consisted of 60% wt. of PE Tipelin polypropylene and 40% wt. of C120 wood flour filler.
In the next stage, a laboratory research stand was prepared, which consisted of four filter columns made of organic glass pipes with an external diameter of 4.4 cm and a length of 100 cm. Columns 1 and 2 were filled with fittings made of a wood-polymer composite, while columns 3 and 4 with polyethylene fittings (Fig. 1). The columns were filled with fittings up to a height of 80 cm. The carriers were divided inside the column into 4 zones, numbered in order from the bottom as zone 1, middle 2 and 3, and the top as zone 4. They were prepared in a manner that allowed to disassemble each of the 4 zones, and then, due to the hinged grid, to take the test fittings out for subsequent studies. The calculated number of fittings placed in the columns is shown in Table 1.

The tested system was fed with wastewater collected from the septic tank (produced by POZPLAST) located in Rybojedzko, Wielkopolska Province, Poznań County, Stęszew Commune (Poland). Assuming that the daily unit water consumption is equal to 0.15 m³/M, the amount of sewage generated by household members was calculated as follows (Table 2):

\[
Q_{dav} = 0.15 \cdot LM \quad \text{– daily water consumption (m³)}
\]

\[
Q_{dmax} = N_{dmax} \cdot Q_{dav} \quad \text{– daily maximum wastewater flow (m³/d)}
\]

\[
Q_{hmax} = Q_{dmax} \cdot N_{hmax}/24 = Q_{dmax}/6 \quad \text{– hourly maximum wastewater flow (m³/h)}
\]

\[
Q_{annual} = Q_{dav} \cdot 365 \quad \text{– annual water consumption for household purposes (m³/y)}
\]

During the research (which lasted for 6 months) bacteriological analyses of raw wastewater from the septic tank and wastewater flowing through the bed were performed. The average wastewater flow rate through the beds was equal to 259.5 cm³/d for the column filled with WPC and 258 cm³/d for a system which contained pure polyethylene fittings.

The microscopic observations of the biofilm formed on the fittings were also carried out using the Delta Optical Evolution 100 Trino Plan LED microscope with a 5x, 10x, 40x and 100x objective and a 12.5x eyepiece. The biofilm observations were most often carried out using a microscope magnification of 62.5x and 125x, and in the case of determining the number of bacteria at a magnification of 500x and 1,250x.

In order to determine the number of bacteria present in the biofilm on the WPC and PE fittings, 3 pieces from zones I and IV of the test columns were collected each month in a sterile manner and placed in a test tube with 10 mL of sterile water. Then, the test tubes with the shaped pieces were shaken, and the obtained suspension was cultured in Petri dishes. After the end of the test cycle, all the pieces from each zone were counted and placed separately in a flask with 100 mL of sterile water. Then the flasks were shaken and the obtained suspension was also cultured in Petri dishes. In the case of testing the bacteria present in the biofilm on fittings, the final result was...
The amount of raw wastewater

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone K</td>
<td>5.4</td>
</tr>
<tr>
<td>Peptone</td>
<td>4.0</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>1.7</td>
</tr>
<tr>
<td>Beef extract</td>
<td>0.4</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>3.5</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1,000.0</td>
</tr>
</tbody>
</table>

Based on the analysis of the test results, it can be concluded that after the passage of raw sewage through columns filled with both WPC and PE fittings, the content of total suspended solids, chemical oxygen demand (COD), total phosphorus and ammonium nitrogen decreased. The amount of total suspended solids in the treated sewage leaving the column packed with WPC was equal to 24 mg/L on average, while after passing through the PE column it was equal to 23 mg/L. These values meet Polish recommendations because the limit value for total suspended solids in treated sewage is 50.0 mg/L. The efficiency of removing the total suspended solids was higher in the columns packed with WPC and amounted to 90.5% compared to the system in which PE fittings were used (88.7%). The COD index in treated sewage amounted to 130.5 mg O₂/L for sewage flowing through the system filled with WPC and 171 mg O₂/L for the PE system. The treatment efficiency of the applied biological deposits was determined at 62.1% for WPC and 50.8% for PE. Taking into account the other quality indicators of treated wastewater, including total phosphorus and ammonium nitrogen, the removal efficiency in case of the former was very comparable for both tested materials (43.3% for WPC and 43.0% for PE). On the other hand, for N-NH₄, the system filled with WPC fittings (52.3%) was more effective than the column filled with PE (29.8%).

The value of the contact angle depends on many factors, for example, the roughness of the surface, its physical and chemical homogeneity, the type of measuring liquid, the type and size of impurities on the surface and the size of the liquid droplets. All these elements may affect the measurement of the contact angle and hinder the interpretation of the obtained results. However, the correct determination of the surface wettability (related to the surface energy of the material) is a useful parameter for assessing the biological interactions between the material and the living organisms. The conducted research allowed for the conclusion that the initial hydrophilicity of the material,
which determines its high wettability, is the conducive factor for the formation of biofilm on the fittings. This results in a good, colonisable adhesion of the microorganisms to the surface of the fittings. In this case, the formation of a biofilm on the surface of the tested materials resulted in an increase of the contact angle. On the basis of the determined value of the contact angle, the nature of the surface of wood composites in the polyethylene matrix was determined and compared with the results obtained for fittings made for pristine polyethylene. It was established that the hydrophilicity of the composite material (91.0°), close to that of pure PE (92.0°), which contributes to the formation of biofilm on the fittings, makes it highly wettable. This results in a good, colonisable adhesion of the microorganisms to the surface of the fittings. After the end of the test, the wettability study was repeated and it was found that the composite fittings exposed to the wastewater became hydrophobic, which proved that the structure of the wood-polymer material was colonized (the contact angle value for the WPC composite was equal to 103.2°) as opposed to the surface of PE fittings (value equal to 92.2°).

In the next stage, microscopic observations were focused on quantitative and qualitative evaluation of microorganisms living on the composites. The determined and described method of testing the samples allowed to obtain all types of microorganisms present on the surface of composites (Figs. 2 and 3), both settled and creeping forms, and also free-floating forms in the case of wastewater analyses. The number of microorganisms was determined based on the modified estimation method using a 5-point scale: very numerous (5 points), numerous (4 points), quite numerous (3 points), not very numerous (2 points) and single (1 points). The highest average number of the found organisms in the entire research period was taken as 100%, while the remaining samples were estimated in relation to the maximum sample. The classification presented in Fig. 4 shows the number of microorganisms in each of the 4 zones of the analysed filter columns.

The number of microorganisms present on the surface of all tested composites with a matrix of PE and pristine PE was notably influenced by ciliates (Ciliata), including Euplotes sp., Litonotus sp., Spirostomum sp., Colpidium colpoda, Amphileptus sp.), rotifers (Rotifera, mainly Rotaria rotatoria) and rhizopoda (rhizopoda, most commonly Arcella vulgaris). This indicates the beneficial effect of the tested solid support on the long-term deposition of various microorganisms (Figs. 5 and 6).

Euplotes sp. was quite numerous in each of the columns in all zones. However, it was the most numerous at WPC. Similar results were observed for Rotaria rotatoria, Litonotus sp. or Amphileptus sp., which were found primarily in columns with WPC fittings. Nematoda appeared in a significant number in each of the columns. These microorganisms which quantitatively dominated in microscopic research. Paramecium bursaria was found in each of the analysed columns. In the WPC column, it was located in zones I, III and IV, it was not observed in zone II, while in the PE column it did not occupy zones II and III.

Analysis of the ratio of microorganisms in individual zones of the studied columns, indicates that among Ciliata small (40–150 µm) and medium-sized (50–400 µm) forms dominated, while among multicellular organisms, the forms were much larger (200 µm–3 mm). These microorganisms are often indicators of good oxygenation and a rather low contamination of wastewater and some forms (e.g., Amphileptus sp., Stylonychia sp.) are rarely found in the sludge. Although Nematoda are found mainly in well-oxygenated sludge, the Rotaria rotatoria is resistant to low oxygen concentration. Bacteria (coccus, bacillus, bacterium, spirillum forms), which play a fundamental role in the process of wastewater treatment, were found in all zones of the
columns, but because they are food for other microorganisms, their number was partially reduced at all stages of wastewater treatment \[38,39\].

The qualitative and quantitative composition of various microorganisms indicates a very large diversity of taxa that are involved in the removal of pollutants contained in wastewater. The most diverse are aerobic and anaerobic bacteria, represented, among others, by *Proteobacteria, Bacteroidetes, Firmicutes, Acidobacteria, Chloroflexi*, including by *Paracoccus* spp., fecal coliforms, *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Vibrio cholerae, Thiothrix* spp., Microthrix spp., Flexibacter spp., *Pseudomonas* spp. *Acinetobacter* spp. [1,13,35,40–43]. There are numerous sedentary, free-swimming and creeping ciliates (*Ciliata*), represented, for example, by: *Epistylis* spp.,

### Table 4

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Total suspended solids (mg/L)</th>
<th>COD (mg/L)</th>
<th>P–og (mg/L)</th>
<th>N–NH₄ (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Raw wastewater</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WPC</td>
<td>253.6</td>
<td>347.0</td>
<td>26.1</td>
<td>97.5</td>
</tr>
<tr>
<td>PE</td>
<td>203.6</td>
<td>347.5</td>
<td>25.8</td>
<td>90.5</td>
</tr>
<tr>
<td><strong>Treated wastewater</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WPC</td>
<td>42.2</td>
<td>130.5</td>
<td>14.8</td>
<td>46.5</td>
</tr>
<tr>
<td>PE</td>
<td>23.0</td>
<td>171.0</td>
<td>14.7</td>
<td>63.5</td>
</tr>
</tbody>
</table>
Epistylis coronata, Epistylis plicatilis, Vorticella convallaria, Vorticella aquadulcis, Carchesium polypinum, Opercularia spp., Metopus spp., Spirostomum spp., Uronema spp., Aspidisca spp., Acineria uncinita, Litonotus spp., Tokophrya spp., Chilodonella spp., Euplotes spp. [7,35,44,45]. The biofilm and activated sludge also includes testate amoebae (Arcella spp., Euglypha spp.), flagellates (Flagellate), sewage fungi, rofers (Rotifera) and nematodes (Nematoda) [7,35,45].

Several such microorganisms have been detected in our research. These organisms have adapted to function under both aerobic and anaerobic conditions. They acclimatize very well in the biofilm on WPC fittings and inhabit various zones.

The biofilms are heterogeneous three-dimensional structures that can contain hundreds of bacterial, fungal and eukaryotic species in the colonizing biofilm. Each of these species interacts through competition for substrates or by predator-prey relationships that cause inherent dynamic changes in biofilm microbiology over time. Principal components analysis showed that the diversity and community-level physiological profiles of microbial communities depended on the working efficiency of the wastewater treatment technologies [2,32].

The conducted analyses indicate the beneficial effect of the tested solid support on the long-term settlement of various microorganisms, which remove contaminants present in wastewater.

Microbiological analysis of raw wastewater flowing into the columns showed a large diversity in the number of psychrophilic bacteria (cultivation at 20°C) at different research dates. Their numbers ranged from 499,000 to 2,381,000 CFU/1 mL (Fig. 7).

The number of bacteria present in the biofilm on fittings from zones I and IV, columns 1 and 2 (filled with WPC fittings) and columns 3 and 4 (filled with fittings from PE) was determined during the same timeframes in which the microbiological quality of wastewater was analysed. The average number of bacteria grown at 20°C per one fitting (CFU/1 fitting) obtained from zones I and IV of columns 1 and 2 (WPC) and zones I and IV of columns 3 and 4 (PE) from three test dates is presented in Fig. 8.

After a comparison of the individual columns, it can be seen that a slightly higher number of bacteria was found on PE fittings. The obtained results indicate that the greatest number of bacteria on the fittings was found when raw wastewater with the highest number of bacteria was supplied to the columns.

After the end of the research cycle and the disassembly of the columns, the average number of bacteria present on the WPC and PE fittings from each zone was calculated. The test results are shown in Fig. 9.

Based on the obtained results, it can be concluded that the average number of bacteria per one fitting (CFU/1 fitting) was most often found on fittings made of WPC (ranging from 27,084,000 for WPC Z I to 49,946,000 for WPC Z III), while on fittings made of PE only higher numbers of bacteria were obtained from the Z I zone (49,754,000 CFU). The bacteria present in the biofilm on the fittings are responsible for the biodegradation of pollutants present in the wastewater flowing through the column, and they are also pray for more organized organisms inhabiting the biofilm, mainly ciliates and rotifers. Not all bacteria are eliminated in the bed, because in the outflow from
the filtration column their number ranged from 226,800 to 2,419,200 CFU/1 mL.

The results described above confirm that the wastewater after the treatment process should be additionally disinfected, as its discharge in this form to the receiving body such as surface water (e.g., a stream, river, lake) may constitute a potential epidemiological threat. The microbes include not only psychrophilic bacteria, but also numerous mesophilic bacteria that originate from humans and warm-blooded animals. Such a danger was pointed out by, for example, [1,5,7,34,40].

4. Conclusions

The conducted studies allowed to establish that the initial hydrophilicity of the material, which determines its high wettability, is the factor that contributes to the formation of biofilm on fittings made of a wood-polymer composite with a composition of 60% wt. of PE Tipelin polypropylene and 40% wt. of C120 wood flour filler. This results in a good, colonization-enabling adhesion of microorganisms to the surface of the fittings used in the model system. In this case, the formation of a biofilm on the surface of the WPC composite resulted in an increase of the contact angle. The composite was also characterized by the highest estimated number of microorganisms. This is mainly due to the properties, structure and physicochemical interactions occurring in the biofilm area, which changes its properties as well as the original properties of the composite material over time. Microorganisms firmly anchored to a solid substrate form a biofilm and trigger numerous reactions in the surrounding environment. The formation of a biofilm on the surface of the composites ultimately resulted in an increase of the contact angle, which was confirmed by repeated studies performed after the end of the tests.

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Conflicts of interest/Competing interests

On behalf of all authors, the corresponding author states there is no conflict of interest.

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