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The influence of polysiloxane functional groups on phototrophic colonization

Joanna Karasiewicz[1]✉, Paulina Nowicka-Krawczyk[2], Rafał M. Olszyński[2], Magdalena Gapińska[3] & Łukasz Majchrzycki[4]

Wood is one of the most widely used construction materials. As a natural material, it is susceptible to the damaging effects of environmental factors. Therefore, researchers are developing innovative solutions to protect wooden surfaces from the harmful effects of moisture or biological agents such as algae and fungi. The objective of the current study was to test the effectiveness of four polysiloxane compounds functionalized with hydrophilic and hydrophobic groups as potential agents for the protection of wood surfaces against biocorrosion caused by photosynthesizing microorganisms. The results of the study clearly indicate that the biological properties of the coating are affected not only by the types of functional groups in the polysiloxane but also by their amounts.

Keywords Organosilicone, Wood, Protective coating, Algae

Abbreviations

PWS25/50	Poly(dimethyl-co-hydromethyl)siloxane
BIKANOL7	Allyl polyether containing seven ethoxy groups with a terminal hydroxyl group
chl	The chlorophyll pigments
^{chl}FI	Fluorescence intensity of chlorophylls
OD_{680}	Inoculation mixtures
OD680 = 3.00	Inoculation mixture with high microalgae density
OD680 = 0.50	Inoculation mixture with low microalgae density

Wood is widely used as a construction material, not only because of its physical properties, such as tensile strength, electrical and heat resistance, and sound absorption, but also because of its natural beauty and capacity to blend harmoniously with the natural world[1,2]. In addition, the use of natural materials such as wood has a positive effect on environmental protection, contributing to the reduction of carbon emissions and mitigating global warming[3,4]. This type of organic material is, however, highly susceptible to biological colonization. Many groups of organisms, such as bacteria, fungi, lichens, and photosynthetic microorganisms, can colonize wooden surfaces, creating different types of biofilms[5,6]. Although the main environmental factors leading to wood decay are UV radiation and water, bacteria and filamentous fungi play a significant role in the biodegradation of wood or wood-based products, directly affecting their structure and leading to their deterioration[7,8]. However, the pioneers in colonizing previously uncolonized surfaces are photoautotrophic microorganisms such as microalgae, due to their limited nutrient requirements, carbon dioxide fixation, and tolerance of a broad range of light, temperature, and moisture levels[9]. Microalgae form 'green', photosynthetic biofilms on wood and wood-based products in all climate zones. As they colonize and grow over the substrate, they not only reduce the aesthetic value of surfaces but also contribute to more serious structural issues. The presence of algal biofilms and crusts affects the moisture retention and thermal conductivity of the substrate, leading to a weakening of the structural matrix of the surface[10,11]. In outdoor conditions, biofilms are complex algae-bacteria consortiums that protect themselves from environmental stressors by secreting extracellular polymeric substances (EPS), which form a slime-like matrix. This increases water absorption and retention, accelerating rotting. Moreover, the EPS can entrap and immobilize airborne hygroscopic compounds (i.e. dust, minerals, aerosols, nutrients

[1]Faculty of Chemistry, Adam Mickiewicz University in Poznań, Uniwersytetu Poznańskiego 8, 61-614 Poznań, Poland. [2]Department of Algology and Mycology, Faculty of Biology and Environmental Protection, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland. [3]Laboratory of Microscopic Imaging and Specialized Biological Techniques, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland. [4]Faculty of Materials Engineering and Technical Physics, Institute of Physics, Poznan University of Technology, Piotrowo 3, 60-965 Poznan, Poland. ✉email: joanna.karasiewicz@amu.edu.pl

and complex organic substances), slowing evaporation even in extremely dry conditions. As a result of the algal cells' shrinkage and expansion during dehydration and rehydration, additional microfractures appear, lowering the durability of the wood. Finally, the dead algal cells serve as nourishment for heterotrophs, allowing further microbial succession[12].

In order to improve the low biodegradation resistance of wood, as well as its high hygroscopicity and low mechanical strength, numerous attempts have been made to develop effective wood modification methods[13–15]. A large number of processes for the preparation of functional wood coatings have been described in detail in numerous papers and comprehensive reviews[5,16,17]. Preservation methods are essential for maintaining the natural properties of wood substrates over time. Many chemical-based methods have been used to control microalgal biofouling, such as Cu/Ag/ZnO nanoparticle polymer coatings, quaternary ammonium salts, and hydrophobic compounds with biocides[12,18,19], but many of them are based on compounds foreign to the surrounding environment[17]. Modified silanes are also commonly used as protective coatings[20–25]. However, the performance of functionalized polysiloxanes as components of protective coatings for wood with anti-biocorrosion properties has not yet been investigated. The objective of the current study was to test the effectiveness of four polysiloxane compounds functionalized with polyether or fluoroalkyl groups (L22, L24, L37 and L38) as potential agents for the protection of wood surfaces against biocorrosion caused by photosynthetic microorganisms.

Functionalized polysiloxanes are obtained by one of three methods: hydrolytic condensation, ring-opening polymerization, or hydrosilylation[26–28]. The first two methods are not selective because they result in a mixture of polysiloxanes with different chain lengths and molecular weights; therefore, the most commonly used method for the synthesis of functionalized polysiloxanes is hydrosilylation. Following hydrosilylation, functional groups can be incorporated into the main siloxane chain or attached to it as side groups.

A total of four different organosilicon derivatives, both mono- and bifunctional, were obtained. Two monofunctional polysiloxanes, one containing a fluoroalkyl group (L38) and the other containing a polyether group (L37) were obtained from typical hydrosilylation reactions. Bifunctional compounds were obtained by subsequent hydrosilylation with two different olefins containing a fluoroalkyl group and a polyether group (L22 and L24). The choice of functional groups was not arbitrary. Despite their specific surface properties, fluoroalkyl groups are chemically unreactive. To take advantage of their hydrophobic nature for surface modification, reactive functional groups must be present in the structure of siloxanes. The presence of a chemically reactive group allows the modifier to bond permanently to the substrate. An allylic polyether containing seven ethoxyl groups with a terminal hydroxyl group was chosen as the second olefin for the study. It should be noted that the presence of ethoxyl groups imparts hydrophilic properties to the olefin.

In conclusion, the results obtained made it possible to determine the effect of the type and number of functional groups with extremely different surface properties on the biological properties of modified wood surfaces.

Materials and methods

Materials

All commercially available chemicals were used as received without any further purification. Poly(dimethyl-co-hydromethyl)siloxane (PWS25/50) was purchased from Gelest (Arlington, VA, USA). Allyl polyether (BIKANOL7) was purchased from ICSO Chemical Production, Kędzierzyn-Koźle. 1,1,2,2,3,3,4,4-Octafluoropentyl allyl ether was synthesized by the Williamson reaction of octafluoropentanol and allyl chloride[29]. The hydrosilylation catalyst was commercially available Karstedt's catalyst purchased from Sigma-Aldrich.

Physicochemical characterization

Magnetic Nuclear Resonance spectra (^{1}H NMR, ^{13}C NMR and ^{29}Si NMR) were taken on a Bruker Ascend 400, at room temperature, with $CDCl_3$ as a solvent. FT-IR spectra were taken on a Nicolet iS20 Mid-Infrared FT-IR Spectrometer with a Gate diamond ATR attachment. The spectra were collected in the range 500–4000 cm^{-1}, with a resolution of 2 cm^{-1}, always recording 32 scans of the background and the sample. The progress of the reaction was quantified by observation of the rate of change in the area of the band with a maximum at 904 cm^{-1}, assigned to the stretching vibrations of Si-H.

Scanning electron microscopy (SEM) analyses were performed using a Quanta FEG 250 (FEI) electron microscope with a beam energy of 10 keV. All samples were analyzed without prior preparation. The microscope was operated in low vacuum mode. Due to the high moisture content of these samples, the wood analyses were performed at a slightly higher pressure of 100 Pa. The energy dispersive spectroscopy (EDS) analyses were performed using the EDS Octane SDD detector (EDAX). The chamber pressure and beam energy were the same as for SEM imaging.

Modification on wood samples

Boards of pine (*Pinus sylvestris* L.) sapwood were cut into pieces (wood samples) measuring 70 mm × 20 mm × 10 mm, which were cleaned with sandpaper and dusted. Reference samples were placed in a vacuum desiccator after cleaning. The remaining samples were subjected to surface modification.The coating was applied by immersing the sample in a 5% solution of the modifier in isopropanol for 30 min. The samples were dried at room temperature for 60 min and then at 60 °C for 12 h. The treated wood samples were stored in a vacuum desiccator. All samples were subsequently subjected to microalgae tests.

Biological material

For the experiment, a non-axenic microalgal culture was prepared. It contained four green algae strains previously isolated from terrestrial biofilms: *Chlorodium saccharophilum* PNK010, *Klebsormidium flaccidum*

PNK013, *Pseudostichococcus monallantoides* PNK037, and *Trebouxia aggregata* PNK080. These algal taxa occur widely in terrestrial photosynthetic biofilms on various types of substrates, both mineral (brick, plaster, glass) and organic (tree bark, technical wood)[30], and in both temperate and tropical regions[12].

To detect the smallest possible changes in the impact of the coating compounds on photosynthetic biofilms, the algal culture was divided into two inoculation mixtures with different microalgal cell densities. The first mixture was high-density ($OD_{680}=3.00$), while the second was low-density ($OD_{680}=0.50$). In nature, a single microalgal cell is carried by the wind to a new surface and can form a new biofilm. Therefore, the low-density inoculation mixture more closely reflects phenomena occurring in the natural environment. At the same time, the high-density mixture demonstrates the potential of a well-developed biofilm to survive on wooden surfaces protected by the compounds.

Seven control wood substrates and seven samples per coating were inoculated with 500 µl of microalgal suspension. The $OD_{680}=3.00$ mixture was applied to six of the samples, while the $OD_{680}=0.50$ mixture was transferred to one.

All wood samples were cultivated in laboratory conditions optimal for microalgal growth (artificial light from fluorescent tubes of 2800 lx in a 16 h/8 h day/night period, with a temperature of 22 °C ± 0.2 °C and humidity of 60% ± 5%) for 7 and 14 days.

The assessment of polysiloxane compound coatings on microalgal biofilm

The impact of biocidal compounds on the viability and survival of photosynthetic microorganisms can be investigated by analysing changes in the most important photosynthetically active pigments – chlorophyll pigments (chl)[31]. Under stress conditions, the fluorescence intensity of chlorophylls (^{chl}FI) decreases, and the concentration of chl-*a*, a type of pigment widely used as an indicator of microalgal biomass, changes.

In this study, the autofluorescence of algal cells was visualized using the Leica TCS SP8 confocal microscope (Lecia Microsystems, Wetzlar, Germany) in the Laboratory of Microscopic Imaging and Specialized Biological Techniques, University of Lodz, and the ^{chl}FI was measured using LAS-AF 3.3.0.10134 software. Chlorophyll fluorescence excitation was induced using a 488 nm White Light Laser, while detection was recorded in the PMT channel at a wavelength of 620–670 nm. Each sample was recorded and scanned along the XYZ axes. The depth with the highest fluorescence pixel count, expressed as grey values [gv], was chosen for the measurements ($n=225$). Wood substrates inoculated with $OD_{680}=3.00$ were analyzed on days 7 and 14 of the experiment ($n=2$ per sample), while those with $OD_{680}=0.50$ were analyzed once, on day 14 ($n=1$ per sample). Dense biofilm samples from wood substrates were collected on day 14 ($n=4$ per sample) for chl-*a* concentration analysis. Algal cells were removed from the substrates with a thin surface layer of wood using a scalpel and transferred to Eppendorf tubes. The pigment was extracted using 96% ethanol following the DIN 38,412 / ISO 10,260 standard and measured in a Spectroquant® Pharo 100 spectrophotometer (Merck, Darmstadt, Germany) with four repetitions for each sample and three repetitions per measurement ($n=12$).

Since the ^{chl}FI data had a non-normal distribution and were independent, the statistical significance ($p \leq 0.05$) of the differences between each sample after 7 and 14 days was checked using the Mann-Whitney U test, while a Kruskal-Wallis one-way analysis of variance by ranks.

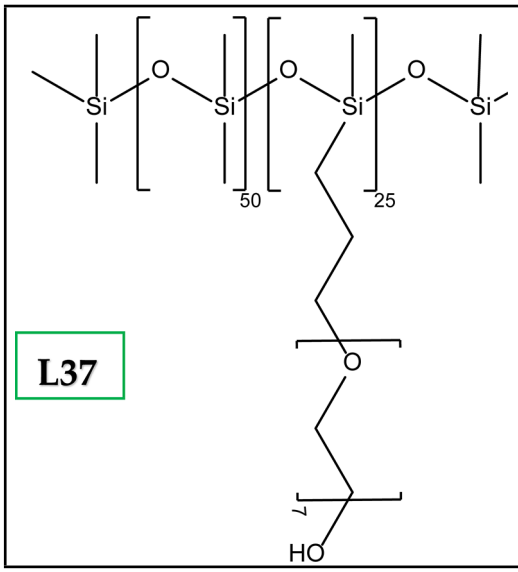
(K-W ANOVA), supported with *post-hoc* Bonferroni correction, was used to correct for multiple comparisons between control and tested samples. The chl-*a* concentration measurements had a normal distribution; therefore, to determine the statistical significance of the difference between the control and test samples, an ANOVA for independent groups with Fisher *post-hoc* correction was used. All statistical analyses were carried out and all figures generated in PQStat v. 1.6.2.

Synthesis of functionalized polysiloxanes (general method)

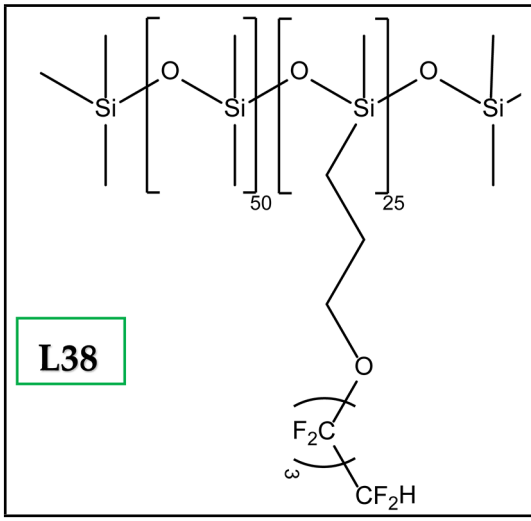
Synthesis of monofunctional polysiloxanes (L37, L38)

Polysiloxanes containing one type of functional group (polyether or fluoroalkyl) were synthesized by the hydrosilylation reaction of poly(dimethyl-co-hydromethyl)siloxane and 1,1,2,2,3,3,4,4-octafluoropentylallyl ether or allyl polyether containing seven ethoxy groups. The process was carried out in the presence of Karstedt's catalyst using toluene as solvent. Since the reaction substrates are not sensitive to moisture, the hydrosilylation reaction could be carried out in an open system, which greatly facilitated the synthesis. An appropriate amount of poly(dimethyl-co-hydromethyl)siloxane, a stoichiometric amount of an olefin, and toluene were placed in a three-necked round-bottom flask equipped with a reflux condenser, a thermometer, and a magnetic stirrer. The mixture was then heated to 110 °C. Karstedt's catalyst, at a concentration of 3×10^{-5} mol Pt per mol Si-H bonds, was added to the reaction flask together with olefin, siloxane and toluene. The reaction was monitored by IR spectroscopy. At the end of the process, the post-reaction mixture was cooled and the products were isolated by distilling off the solvent and excess olefin under reduced pressure. The pure product was obtained in high yields of 96–98%. The products were subjected to spectroscopic analysis to verify that the hypothesized structure had been synthesized (S1).

Product characterization:

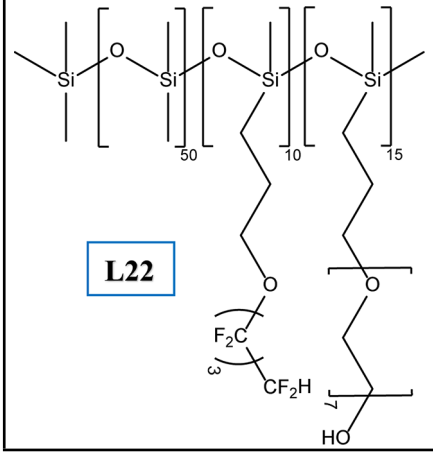


^{1}H NMR ($CDCl_3$) δ (ppm) : 0,13 (375 H, $[Si(CH_2)CH_3]$; $Si(CH_3)_2$); 0,20 (18 H $Si(CH_3)_3$); 0,59 (50 H, $SiCH_2$); 1,46 (50 H, $SiCH_2CH_2$); 3,37 (50 H, $SiCH_2CH_2CH_2$); 3,53 (350 H, OCH_2CH_2); 3,64 (350 H, OCH_2CH_2); 5,40 (25, OH)
^{13}C NMR ($CDCl_3$) δ (ppm) : 4,52 ($Si(CH_2)CH_3$); 5,29 ($Si(CH_3)_2$); 5,81 ($Si(CH_3)_3$); 17,23 ($SiCH_2CH_2$) 22,3 ($SiCH_2$); 61,33 (OCH_2CH_2); 70,32 (OCH_2); 73,75 ($SiCH_2CH_2CH_2$)
^{29}Si NMR ($CDCl_3$) δ (ppm) : -22,03 ($OSi(CH_3)_2$); -22,63 ($OSiCH_3$)

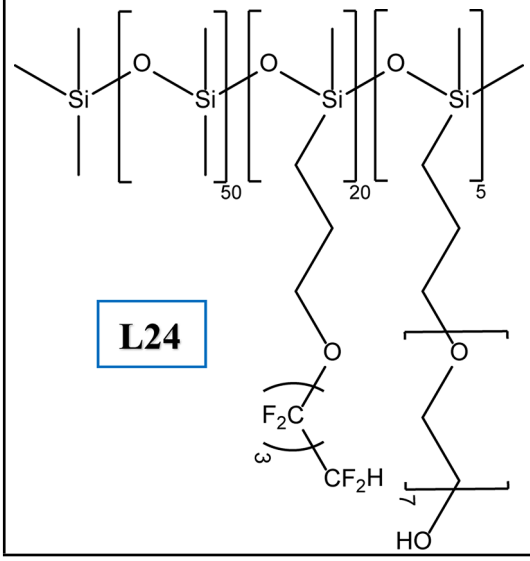


^{1}H NMR ($CDCl_3$) δ (ppm) : 0,11 (375 H, $[Si(CH_2)CH_3]$; $Si(CH_3)_2$); 0,21 (18 H $Si(CH_3)_3$); 0,59 (50 H, $SiCH_2$); 1,51 (50 H, $SiCH_2CH_2$); 3,42 (50 H, $SiCH_2CH_2CH_2$); 6,39 (25 H, CF_2H)
^{13}C NMR ($CDCl_3$) δ (ppm) : 4,51 ($[Si(CH_2)CH_3]$; 5,30 ($Si(CH_3)_2$); 5,85 ($Si(CH_3)_3$); 17,49 ($SiCH_2CH_2$) 22,66 ($SiCH_2$); 60,60 ($SiCH_2CH_2CH_2$); 111,96 ($OCF_2CF_2CF_2$); 117,82 (CF_2H); 118,77 ($OCF_2CF_2CF_2$); 122,99 ($OCF_2CF_2CF_2$)
^{29}Si NMR ($CDCl_3$) δ (ppm) : -21,78 ($OSi(CH_3)_2$); -22,86 ($OSiCH_3$)

Synthesis of polysiloxanes containing mixed functional groups (L22,L24)
Polysiloxane containing 25 Si-H bonds was used in the synthesis. Siloxanes containing fluorofunctional groups and polyether reactive groups were synthesized by the hydrosilylation reaction of poly(dimethyl-co-hydromethyl)siloxane and 1,1,2,2,3,3,4,4-octafluoropentylallyl ether followed by allyl polyether containing seven ethoxy groups with a terminal hydroxyl group. The process was carried out in the presence of Karstedt's complex as a catalyst. In the first step, poly(dimethylco-)siloxane and 1,1,2,2,3,3,4, 4-octafluoropentyl allyl ether and toluene were placed in a three-necked round-bottom flask equipped with a thermometer, a reflux condenser and a magnetic stirrer. Karstedt's catalyst (3×10^{-5} mol Pt per mol Si-H) was then added at room temperature. After the introduction of the catalyst, the solution was heated to a temperature of 110 °C. When complete conversion of the fluorinated olefin had occurred (as monitored by FT-IR analysis), an appropriate amount of the second olefin, allyl polyether, was added. After all the reagents had been introduced, the solution was kept under the same conditions as before. The course of the reaction was monitored by IR spectroscopy, by observing the disappearance of the band at 904 cm^{-1}, assigned to the Si-H bond in the substrate. At the end of the process, the post-reaction mixture was cooled and the products were isolated by distilling off the solvent and excess olefin under reduced pressure. The pure product was obtained in a high yield of 97–98%, (S2). NMR analysis of the products confirmed their structure.
Product characterization:



^{1}H NMR ($CDCl_3$) δ (ppm) : 0,07 (375 H, [$Si(CH_3)CH_3$]; $Si(CH_3)_3$); 0,21 (18 H $Si(CH_3)_3$); 0,47 (50 H, $SiCH_2$); 1,47 (50 H, $SiCH_2CH_2$); 3,36 (50 H, $SiCH_2CH_2CH_2$); 3,57 (210 H, OCH_2CH_2), 3,66 (210 H OCH_2CH_2), 5,5 (15 H, OH); 6,38 (10 H, CF_2H)
^{13}C NMR ($CDCl_3$) δ (ppm) : 4,5 ($Si(CH_3)CH_3$); 5,42 ($Si(CH_3)_2$); 5,8 ($Si(CH_3)_3$); 16,18 ($SiCH_2CH_2CH_2$), 22,49 ($SiCH_2CH_2CH_2$); 61,35 (OCH_2CH_2) 61,47 ($CH_2O(CF_2)_3CF_2H$); 70,38 (OCH_2CH_2); 111,05 ($OCF_2CF_2CF_2CF_2H$); 117,84 ($OCF_2CF_2CF_2CF_2H$); 120,87 ($OCF_2CF_2CF_2CF_2H$); 126,18 ($OCF_2CF_2CF_2CF_2H$)
^{29}Si NMR ($CDCl_3$) δ (ppm) : 7,30 ($Si(CH_3)_3$); -21,84 ($Si(CH_3)_2$); -57,82 ($Si(CH_2)CH_3$)



^{1}H NMR ($CDCl_3$) δ (ppm) : 0,13 (375 H, [$Si(CH_2)CH_3$]; $Si(CH_3)_2$); 0,20 (18 H $Si(CH_3)_3$); 0,51 (50 H, $SiCH_2$); 1,40 (50 H, $SiCH_2CH_2$); 3,36 (50 H, $SiCH_2CH_2CH_2$); 3,71 (70 H, OCH_2CH_2), 3,66 (70 H, OCH_2CH_2), 5,52 (5 H, OH); 6,28 (20 H, CF_2H)
^{13}C NMR ($CDCl_3$) δ (ppm) : 4,17 ($Si(CH_2)CH_3$); 5,4 ($Si(CH_3)_2$); 5,91 ($Si(CH_3)_3$); 16,31 ($SiCH_2CH_2CH_2$), 22,45 ($SiCH_2CH_2CH_2$); 62,02 (OCH_2CH_2) 62,17 ($CH_2O(CF_2)_3CF_2H$); 70,36 (OCH_2CH_2); 111,15 ($OCF_2CF_2CF_2CF_2H$); 117,62 ($OCF_2CF_2CF_2CF_2H$); 121,08 ($OCF_2CF_2CF_2CF_2H$); 126,34 ($OCF_2CF_2CF_2CF_2H$)
^{29}Si NMR ($CDCl_3$) δ (ppm) : 7,21 ($Si(CH_3)_3$); -21,96 ($Si(CH_3)_2$); -61,32 ($Si(CH_2)CH_3$)

Results

Assessment of the effects of polysiloxane compound coatings on the microalgal biofilm

During the experiment, the photosynthetic activity of cells in the control sample with a high-density biofilm increased over time (Fig. 1). Microalgal cells adapted well to the wood surface and the biofilm remained physiologically active. Moreover, there was no statistically significant difference in ^{chl}FI between the low- and high-density control biofilms after 14 days of cultivation (Table 1).

After 14 days of cultivation, the ^{chl}FI of the high-density biofilms growing on wood substrates treated with L24 and L38 did not differ significantly from that of the control. However, the median values changed over time, decreasing by 26% for L24 and increasing by 95% for L38 (Fig. 1). The ^{chl}FI of samples with the low-density inoculation mixture and thin biofilms differed significantly from that of the control for both compounds after 14 days of cultivation, and also differed significantly from that of the respective high-density biofilm samples (Fig. 2; Table 1). The median ^{chl}FI in both low-density samples (L24 and L38) was similar – approximately 97 gv, which was 44% lower than the control. Nevertheless, there were some cells in both biofilms with high physiological activity, comparable to that observed for the control. The maximum ^{chl}FI for L38 was equal to the control at 250 gv, while for L24 it was slightly lower at 237 gv.

A significant change in the physiological activity of microalgal cells from high-density inoculations growing on wood substrate was noted after 14 days of cultivation in the case of two polysiloxane compounds – L22 and L37. In the case of the first compound, median ^{chl}FI decreased by 53% relative to that of the control, and the change was maintained at a similar level for both the median and maximum value. The second compound was more effective, as the median ^{chl}FI decreased by 85% relative to that of the control and was constant over time; however, the maximum value decreased after another 7 days of cultivation from 106 to 77 gv.

The effect of L22 and L37 on algal cells from low-density inoculations was even stronger than their effect on cells from high-density innoculations. In CLSM images, cell autofluorescence was noticeably weaker for low-density than for high-density biofilm samples (Fig. 3). The statistical differences between the ^{chl}FI of high-density and low-density biofilm samples growing on both compound substrates were significant.

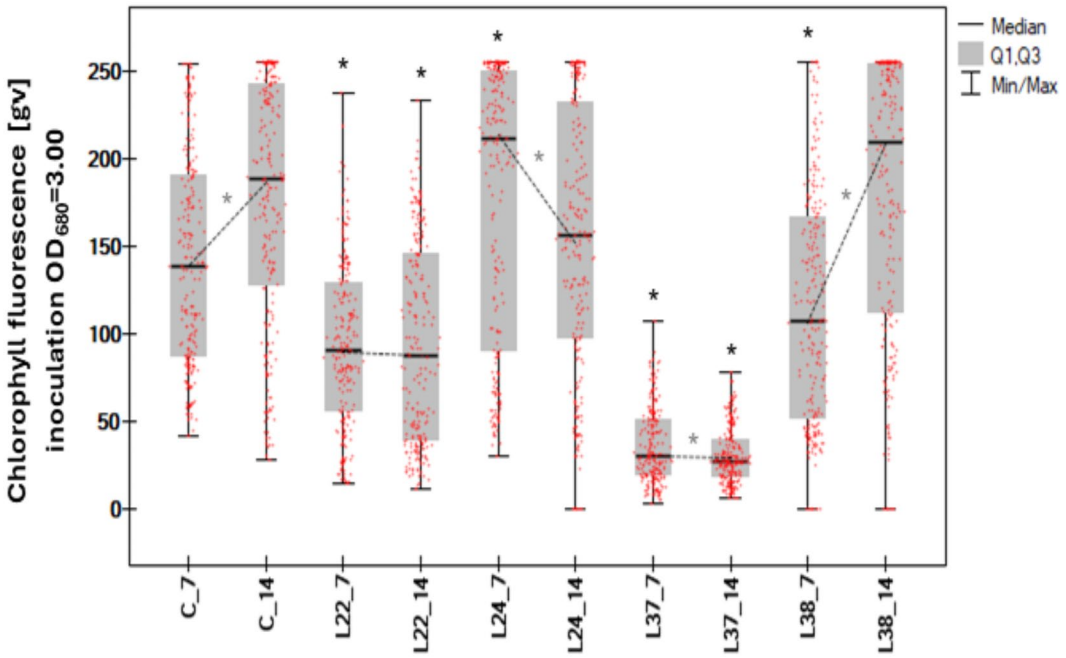


Fig. 1. Changes in the ^{chl}FI of microalgal cells ($OD_{680} = 3.00$) on a plain wood substrate (control) and wood substrates coated with polysiloxane compounds (treated samples) after 7 and 14 days of cultivation; grey asterisks indicate the statistical significance ($p \leq 0.05$) of changes within each sample with time, while black asterisks indicate the statistical significance ($p \leq 0.05$) of the differences between tested samples and control in each week of the experiment.

p values		$OD_{680} = 3.00$				
		C_14	L22_14	L24_14	L37_14	L38_14
$OD_{680} = 0.50$	C_14	1	$< 1 \times 10^{-6}$	1	$< 1 \times 10^{-6}$	1
	L22_14	$< 1 \times 10^{-6}$	$< 1 \times 10^{-6}$	$< 1 \times 10^{-6}$	1	$< 1 \times 10^{-6}$
	L24_14	$< 1 \times 10^{-6}$	1	$< 1 \times 10^{-6}$	$< 1 \times 10^{-6}$	$< 1 \times 10^{-6}$
	L37_14	$< 1 \times 10^{-6}$	$< 1 \times 10^{-6}$	$< 1 \times 10^{-6}$	1	$< 1 \times 10^{-6}$
	L38_14	$< 1 \times 10^{-6}$	0.364	0.001	$< 1 \times 10^{-6}$	$< 1 \times 10^{-6}$

Table 1. The statistical significance (p values) of the differences between the ^{chl}FI of the high-density ($OD_{680} = 3.00$) and low-density ($OD_{680} = 0.50$) control and polysiloxane-coated samples after 14 days of cultivation.

Although the median ^{chl}FI in both low-density biofilms was similar – 29 gv for L22 and 20 gv for L37 – there was a considerable difference between the maximum ^{chl}FI of the two samples. For L22, the maximum ^{chl}FI was 45% lower than for the control, while for L37, it was 73% lower.

After 14 days of incubation, the low-density algal mixture on the protected wood samples grew in the form of a thin biofilm composed of only a few layers of cells; therefore, the 3D CLSM visualization showed quite a similar effect in the cross-section of the biofilms (Fig. 4). However, where a high-density inoculation mixture had been applied, algal cells grew over the samples in the form of multilayered biofilms. After 14 days of algal incubation on the control and L38 compound-coated wood substrates, there was no significant change in the autofluorescence of the samples (or, correspondingly, the depth of the biofilms) in the cross-sectional view. For L37, the most effective compound in decreasing the ^{chl}FI value, autofluorescence was completely absent in the cell layers in contact with the surface of the substrate.

The initial chl-*a* concentration of the $OD_{680} = 3.00$ inoculation mixture was 53.8 µg/L. After 14 days of algal incubation on the control wood sample, it increased to an average of 78.9 µg/L (Fig. 5). The same tendency was observed in the case of samples growing on two of the polysiloxane-coated wood samples – L24 and L38. In these cases, the biomass of algal cells, expressed by chl-*a* concentration, increased. For L24, the average was higher than for the initial inoculation mixture by 10.2 µg/L, while for L38 it was higher by 15.4 µg/L. Nevertheless, the final algal biomass values for the two treated substrates were lower than that of the control by 23% and 12% respectively, and the change in the chl-*a* concentration was statistically significant. Only in the case of the L22- and L37-coated substrates did the average chl-*a* concentration decrease after 14 days of inoculation, to 28.4 µg/L

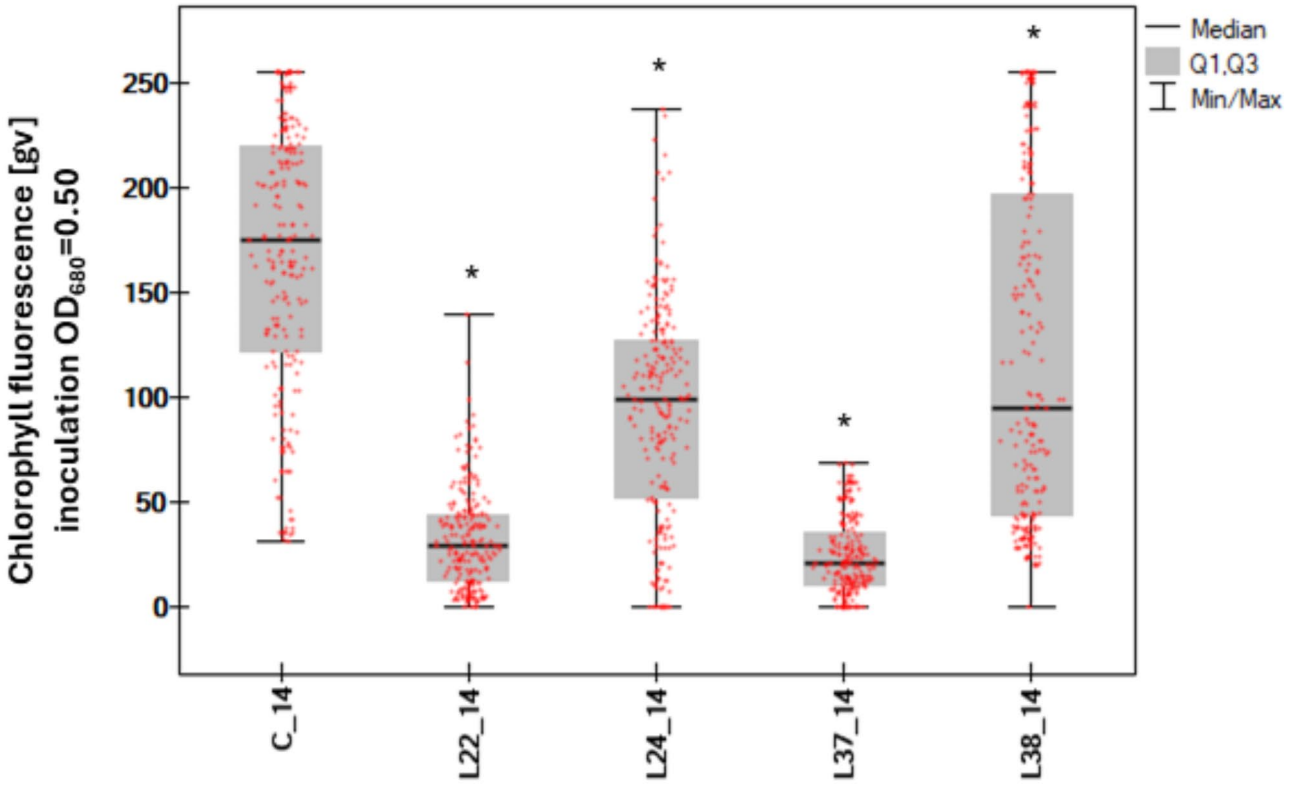


Fig. 2. Changes in the ^{chl}FI of microalgal cells ($OD_{680} = 0.50$) on a plain wood substrate (control) and wood substrates coated with polysiloxane compounds (treated samples) after 14 days of cultivation; the black asterisks indicate the statistical significance ($p \leq 0.05$) of the differences between the treated samples and the control.

for L22 and 15.8 µg/L for L37. At the end of cultivation, the algal biomass values of the samples were lower than that of the control by 64% and 80% respectively.

Scanning electron microscopy analysis

The analysis of the protective properties of the coatings was carried out using Scanning Electron Microscopy (SEM) with a beam energy of 10 keV, operated in low vacuum mode at a pressure of 100 Pa. The control sample of wood without any protective coating had a typical wood structure with clearly visible xylem vessels covered by algal colonies and occasionally visible uncovered tracheids (Fig. 6). The higher magnification images show oval structures of cells a few microns in diameter connected by biofilm to the well-developed network of algae. The wood surface covered by L22 shows some areas of xylem vessels that are not covered by algal colonies, confirming the substance's protective properties. It can clearly be seen that the structure of the algal cells has not been disturbed. However, it should be noted that the cells are not connected by the biofilm. Like L22, L24 did not disrupt the algal cell structure, and the biofilm is not present. However, the area of wood uncovered by the algal colony is slightly smaller than in the case of L22, indicating lower protective activity. The highest protective activity can be observed in the case of L37, as the majority of the wood surface was free of algae after treatment with this compound. Moreover, the remaining algal cells form only small colonies separated by the bare wood surface. It is also worth mentioning that highly deformed, rough particles with a diameter of a few micrometers can be clearly seen. It is reasonable to assume that such structures are dead algae cells. In contrast, L38 offers the least protection. After treatment with this compound, the algal cells were undisturbed, the biofilm was clearly visible, and the only protective effect manifested itself in tiny areas of wood that were not covered by algal colonies.

Discussion

In terrestrial environments, atmospheric conditions such as wind and rain are essential for the transfer of microalgae from one substrate to another[32,33]. Individual cells detach from well-developed biofilms and are dispersed by the wind to new substrates. When they reach new surfaces, the extracellular polymeric substance they produce helps them to adhere to even very smooth surfaces, such as glass. Under appropriate humidity, they begin to develop multi-layered biofilms composed of bacterial-algal matrix that can survive the harsh conditions of the terrestrial environment[34]. For this experiment, all inoculation mixtures were composed of selected algal strains with their native bacterial microflora. By using this non-axenic culture, it was possible to be more confident that the algae would adapt to the wood substrates and form biofilms. Moreover, if a positive anti-algal

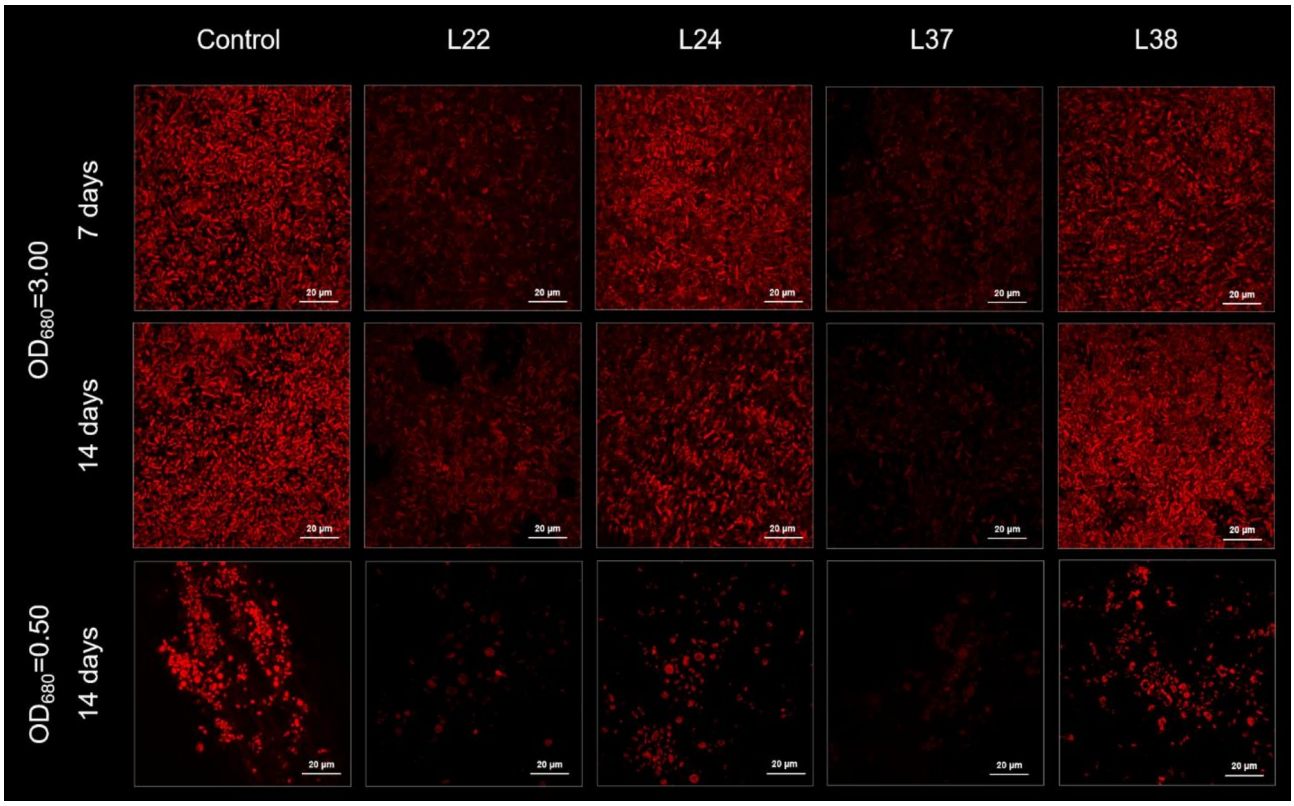


Fig. 3. The CLSM micro images of chlorophyll fluorescence in algal biofilms growing on a wood substrate coated with polysiloxane compounds; scale bar 20 µm.

effect occurs, this type of culture is more likely to reflect ongoing phenomena in the environment where bacteria and algae cooperate in the formation and survival of photosynthetic biofilms. For both the high- and low-density inoculation mixtures, the control wood substrate was overgrown with algal cells and biofilms were formed.

Many studies deal with the problem of microbial colonization of building materials and search for effective antifouling agents. They emphasize the role of hydrophobic substances such as alkyl-silanes and alkyl-alkoxy-siloxanes in reducing cell adhesion; however, their affinity for non-polar substances may reduce their effectiveness, especially when dealing with organic fouling[35–37]. Terrestrial microalgae are poikilohydric microorganisms, which means that they cannot regulate the water balance in their cells, so their metabolism is highly dependent on the humidity of the environment[38]. In the case of non-smooth, porous or (like wood) fibrous materials, when algal cells are transferred to the surface, they can nest in surface irregularities and proliferate despite the presence of anti-adhesive agents[39]. In addition, hydrophobic coatings prevent water infiltration into the substrate, and under high humidity, water droplets remain in the surface irregularities for longer periods of time, making them more available to algal cells. On the other hand, coatings containing compounds with polar groups in their structure increase the moisture content of the substrate, and some reports indicate that algae prefer to adhere to hydrophilic materials[39]. However, reports show evidence of the opposite tendency, where microalgal colonization on superhydrophilic surfaces was 50% lower than on superhydrophobic surfaces[40].

In this experiment, four polysiloxane compounds differing in structure and surface properties were evaluated for their anti-algal properties. Two of them – L22 and L37 – exhibited a positive effect, with a slightly higher efficacy in suppressing algal photosynthetic activity in the case of L37. Both compounds have hydroxypolyether groups in their structure, but in different numbers – L37 has 25 chains, while L22 has only 15 chains. In addition, L22 was also functionalized with 10 fluoroalkyl chains. Since the two other compounds tested (L24 and L38) did not prevent an increase in algal biomass and chl-a concentration after 14 days of incubation and were also functionalized with fluoroalkyl chains in increasing numbers (20 and 25 chains, respectively), it is obvious that this modification does not play a significant role in the reduction of cell viability. Looking at the percentage of chlFI suppression and chl-a concentration reduction in algal cells growing on wood protected by polysiloxane compounds functionalized with hydroxypolyether groups, there is a clear dependence on the number of hydroxypolyether groups and the anti-algal properties. L37, with 25 chains (reduction in maximum chlFI by 73% and decrease in chl-a by 80%), is more effective than L22, with 15 chains (reduction in maximum chlFI by 45% and decrease in chl-a by 64%), and both are more effective than L24, which has 5 chains (reduction in maximum chlFI by 5% and decrease in chl-a by 24%). L38, which lacks hydroxypolyether groups, did not have a statistically significant effect on the chlFI of a well-formed, dense biofilm and only led to a very small decrease in chlFI in the case of a low-density inoculation mixture; however, the algal biomass on wood protected with L38 was lower than that on the control (12% lower chl-a concentration) after 14 days of incubation. Summary

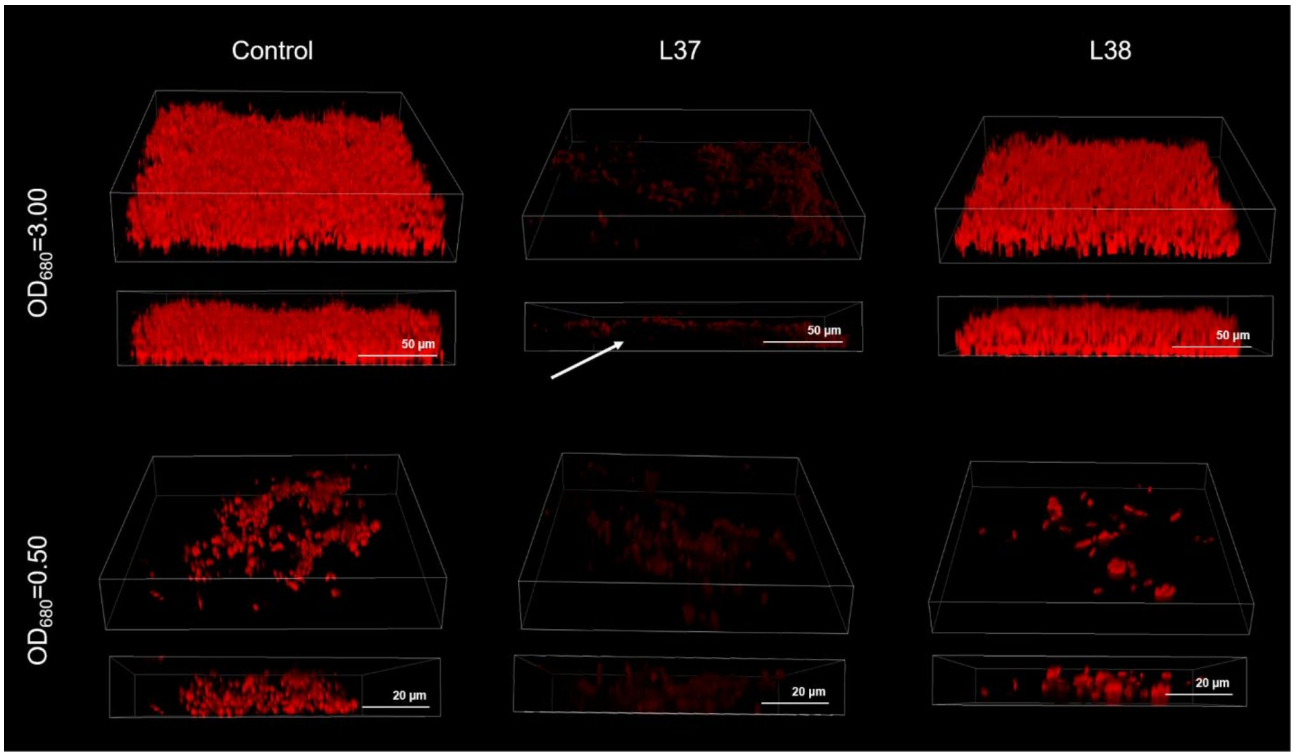


Fig. 4. The 3D CLSM micro images of algal biofilms in cross-section after growing for 14 days on a wood substrate coated with the most and least effective polysiloxane compounds; the arrow highlights the absence of autofluorescence in the high-density biofilm in contact with the surface of the wood treated with L37; scale bar 50 µm and 20 µm.

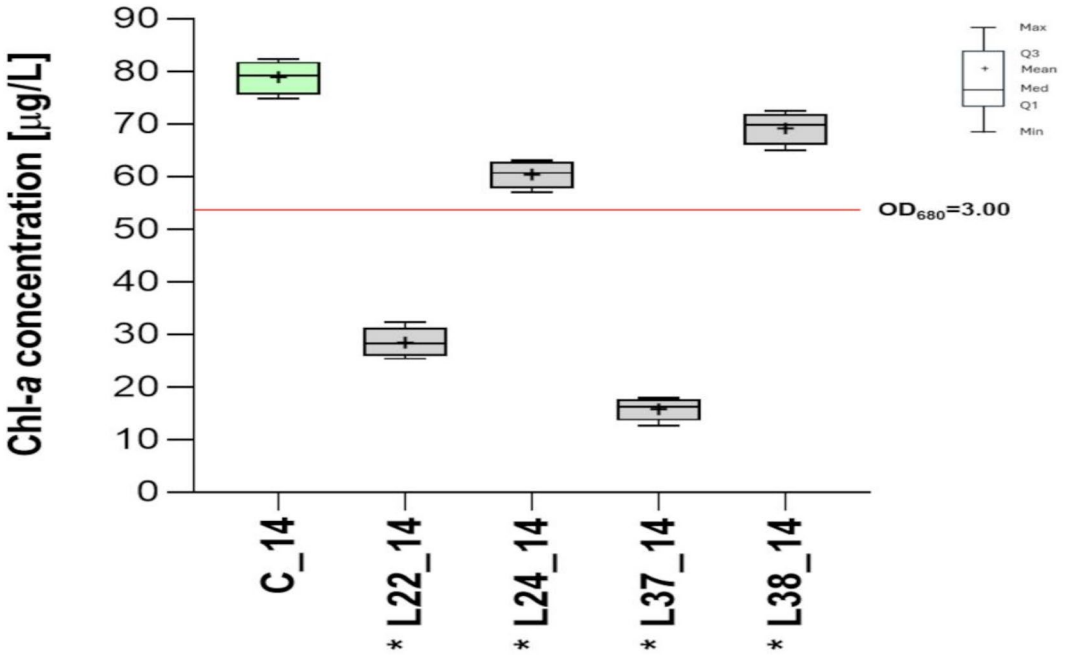


Fig. 5. Changes in the chl-*a* concentration of the microalgal biofilm for the control wood substrate and the samples coated with polysiloxane compounds after 14 days of cultivation; black asterisks indicate the statistical significance ($p \leq 0.05$) of the differences between the samples and the control, while the solid red line shows the chl-*a* concentration of the inoculation mixture.

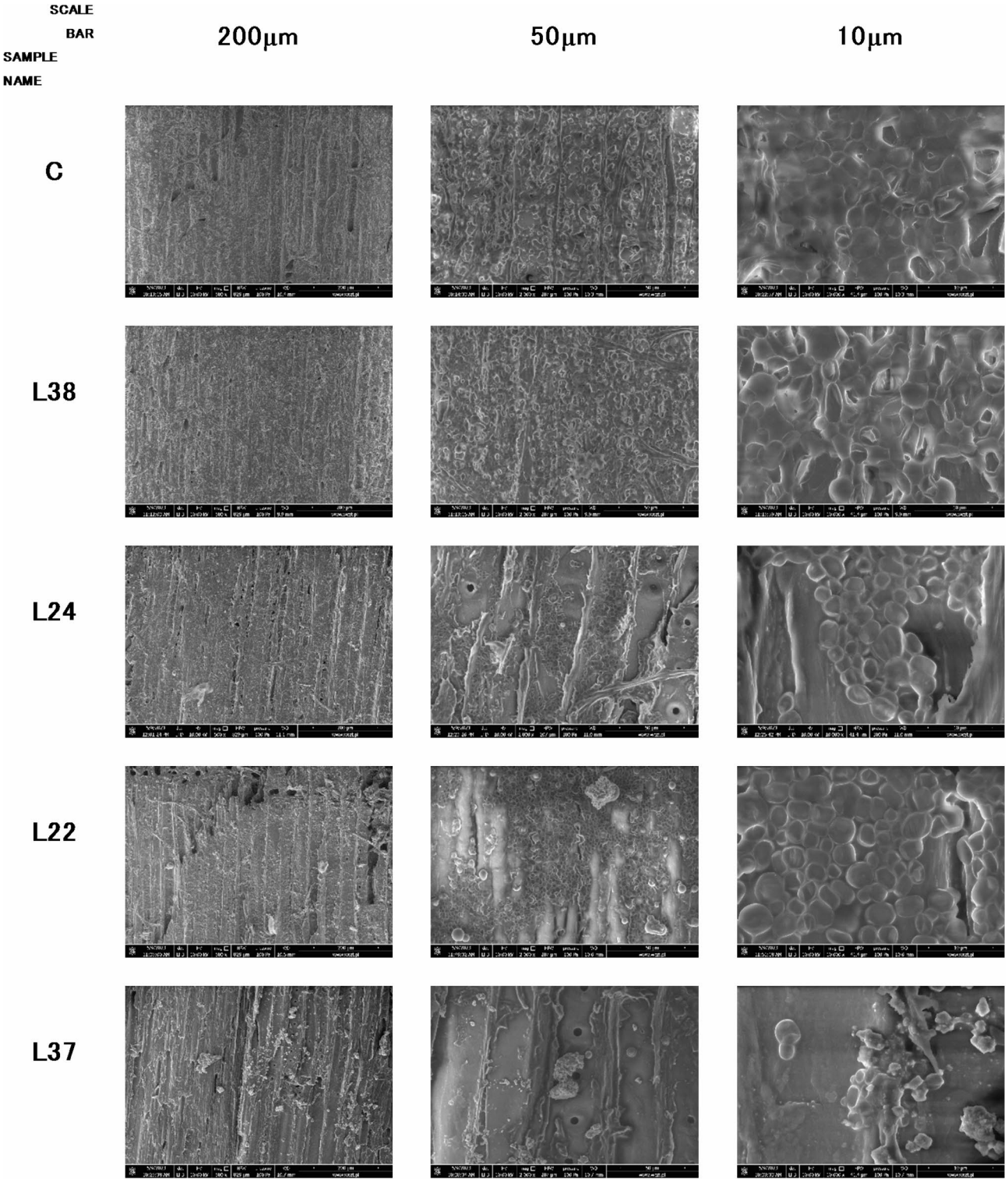


Fig. 6. SEM photomicrographs of microalgal biofilms after 14 days of growth on wood: Control (C), L38, L24, L22, L37 coating. Scale bar: 200 µm, 50 µm and 10 µm.

(Fig. 7), the results of the research clearly indicate that not only the type of functional groups in the polysiloxane, but also their quantity influences the biological properties of the coating.

Chlorophyll a concentration and chlorophyll fluorescence may not always change in tandem. The photosynthetic activity of cells, which is directly related to fluorescence intensity, may remain high even if the biomass of cells, expressed in terms of pigment concentration, is decreasing. The frequency of cell division in biofilms under stress conditions may slow down, which directly affects the total pigment concentration.

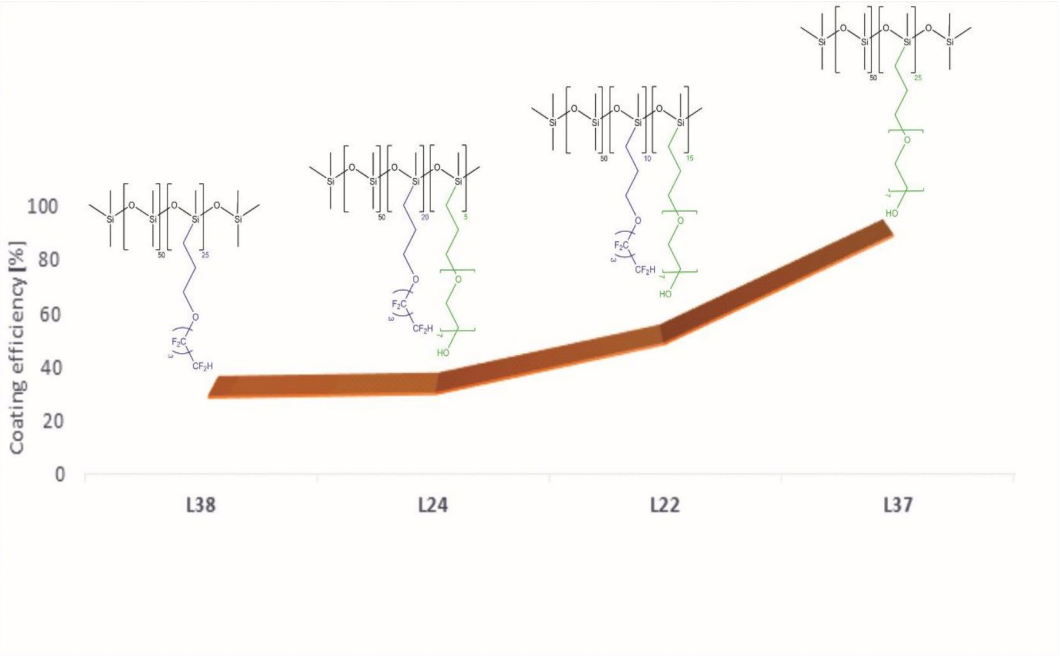


Fig. 7. Curve of the effect of the number and type of functional groups in the polysiloxane on the biological properties of the coating.

However, many cells may still be physiologically active. Moreover, chlorophyll fluorescence may even increase under the effect of active compounds, because the photosynthetic metabolism of cells increases as they adapt to the negative effect of compounds[31]. In the case of desiccation stress, a physiologically dangerous and long-lasting cell degradation factor, chlorophyll fluorescence always changes[41].

Microalgal cells growing on substrates coated with hydrophilic compounds exhibit the morphological and physiological symptoms of desiccation[29]. The number of hydroxypolyether groups in the polysiloxane coatings tested had the greatest impact on the physiological response of the cells. More hydrophilic chains results in higher quenching of chlorophyll fluorescence and a reduction in the photosynthetic activity of cells. It also directly affects the concentration of chlorophyll pigments. Hydrophilic compounds increase the rate of microalgal desiccation. Damage to cells under this type of stress not only leads to pigment degradation but also decreases the rate of cell division in photosynthetic organisms[42]. The most sensitive sites in the photosynthetic apparatus of aerophytic green algae, such as photosystem II (PSII) with its oxygen-evolving complex, as well as the ATP-generation and carbon assimilation processes, quickly exhibit symptoms of dysfunction. Photosynthesis is usually completely blocked in a dehydrated state, and even if excitation energy is still absorbed, it cannot be used for electron transport, leading to photoinhibition and photodamage of cells. Although green algae are known for their resistance to desiccation stress, this physiological state also limits the supply of carbon dioxide for carbon fixation and, ultimately, disrupts repair mechanisms by inactivating repair-protein translation[43].

Conclusion

The aim of the research was to test the effectiveness of four polysiloxane compounds functionalized with polyether (L37), fluoroalkyl (L38) or both groups (L22 and L24) as potential surface protection agents for wood against biocorrosion by photosynthetic microorganisms. By introducing two substituents with extreme surface properties—hydrophilic (polyether groups) and hydrophobic (fluoroalkyl groups)—compounds with amphiphilic properties were obtained. The results of the study clearly indicate that not only the type of functional groups in the polysiloxane but also their quantity influences the biological properties of the coating (Fig. 7). The study shows that the presence of hydrophilic groups in the coating has a positive effect on the protection of wood against algae. It was also found that the higher the ratio of hydrophilic to hydrophobic groups, the better the protection. However, the best results were obtained with the monofunctional compound L37, whose molecules contain only polyether groups. Thus, it was shown that the hydrophilicity, and not the hydrophobicity, of the substituents determines the effect of biological corrosion inhibitors on wood.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Author contributions
Conceptualization, JK; Writing—Original Draft Preparation, JK, PNK, and RO; Investigation, JK; Formal Analyses, JK, PNK, MG and ŁM; Methodology, JK and PNK; Software, JK, PNK, and RO; Investigation, RO and ŁM; Resources, JK and PNK; Data Curation, JK and PNK; Visualization, JK and RO; Writing—Reviewing and Editing, JK and PNK.

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Declarations

Competing interests
The authors declare no competing interests.

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Correspondence and requests for materials should be addressed to J.K.

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