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BIO-TREATMENT OF SPRUCE WOOD FOR IMPROVING OF ITS PERMEABILITY AND SOAKING PART 1: DIRECT TREATMENT WITH THE BACTERIUM *BACILLUS SUBTILIS*

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ABSTRACT

Impregnability of spruce wood is very difficult due to aspiration of pit membranes in tracheids, mainly in early wood. One way of how to keep the pit membranes permanently in an open state is their biodegradation by some bacteria - for example by *Bacillus subtilis*. For the experiment we used one healthy green spruce log from which sapwood and heartwood specimens have been immediately prepared. Biological attack of these specimens was carried out in glass vessels filled with distilled water and culture of the bacterium *B. subtilis* at a hydro-module of 1 : 5, at temperatures of 20 or 30 °C, during 1, 3, 6 or 9 weeks. Bacterial culture was charged to the media either only at the beginning of the experiment, or every other week. One group of sap- and heartwood specimens was simultaneously ponded for four months in non-sterile water. Microscopic analyses showed that pit membranes in the sapwood are obviously totally opened after the biological attack. The permeability and kinetics of water soaking of the bacterially attacked sapwood specimens increased more apparently than of those soaked for 4 months in non-sterile water. However, the intentional bacterial attack did not improve the heartwood impregnability.

KEY WORDS: spruce, *Bacillus subtilis*, torus opening, permeability, soaking

INTRODUCTION

Efficacy of the chemical protection of wood with biocides and other preservatives depends on their sufficient penetration into wood. Transport of preservatives into wood depends on more factors: - structural characteristic of wood and its moisture, - physical and chemical properties of transported substances, - pressure, diffusion, or other driving forces (Reinprecht 1993). Conifers belong to wood species which are very difficult to impregnate due to aspiration of their pit membranes at the drying process (Bolton and Petty 1978, Siau 1984, Usta 2005).

In practice, it is sometimes necessary to do suitable pre-treatments of spruce, fir and other coniferous poles, mine commodities, etc. before their impregnation with the aim to achieve a greater depth of penetration and a higher retention of the preservative. Various methods of pre-treatment are used to improve impregnability of coniferous woods (Kurjatko and Reinprecht 1993, Hansmann et al. 2002). Permanently the most used are the mechanical treatments concerned with various ways of cutting and incising of wood to achieve an increase of its front surfaces (Morrel and Morris 2002). Other methods are physical and physical-chemical pre-treatments using drying processes for making microscopic cracks (Booker and Evans 1994, Moldrup 1995), laser beams for increasing the area of front surfaces (Reinprecht and Horský 1990, Goodel et al. 1991, Komora et al. 1992), ultrasound waves during impregnation (Marčok and Kurjatko 1987), or acetylation of –OH groups in pit membranes (Rapp et al. 2000). Pit membranes can be opened also by hydrothermal (Nicholas and Thomas 1968) and chemical pre-treatments (Militz and Homan 1993).

Biological pre-treatments of conifers were analysed in more experimental works, as well. There are used certain species of bacteria and fungi which increase permeability of wood at minimal disintegration of its cell walls. For this purpose, these organisms produce specific enzymes only of hydrolyse type (Blanchette et al. 1990, Schmidt 2006).

Improvement of the permeability of spruce and pine poles by a long-term ponding in non-sterile water, that is often explained by the influence of bacteria to torus, has been known for a longer time (Ellwood and Eckland 1959, Unligil 1972, Peek and Liese 1979, Singh et al. 1998). For example, Unligil (1972) observed the increase of spruce sapwood impregnability with creosote by 155 % after its 5 or 9 weeks of ponding in natural pool. The sapwood was fully impregnable but permeability of the heartwood did not change. The modulus of rupture was lower by 4 % after this treatment. Singh et al. (1998) examined the influence of wood ponding in non-sterile water to the depth of penetration of acrylate applied by painting on the boards of pine *Pinus radiata*. The boards were ponded in periods from 2 to 12 weeks, dried and then painted with a basic paint. At the control (not ponded) specimens there was observed penetration to the depth of two cells. After 2 weeks of ponding it was already to the depth of 5 – 6 cells, and by ponding longer than 4 weeks it was more than 10 cells. These changes were explained by the degradation influence of bacteria on the membrane of pits. Dunleavy and McQuire (1970) have also observed a high increase of the permeability in sap zone of *Picea sitchensis* and *Picea abies* woods after their 6 or 8-week ponding. It was found out that increase of permeability was caused either by damage of toruses and also due to damage of parenchyma cell membranes. Banks (1970) examined how to limit the escape of creosote oil from pine poles after Rüping impregnation cycle. He increased the permeability of pine poles by spraying them with water containing active bacteria. Bacterial attack caused degradation of pits. Satisfactory results were achieved after 3 or 4 weeks at a temperature of 15 °C. Efransjah et al. (1989) analysed spruce wood after 5 month of ponding in the presence of bacterium *Bacillus subtilis* from the point of view of the permeability and changes of the mechanical properties. They found out improving of the permeability in lengthwise direction in sap zone of spruce wood caused by the attack of bacteria to the toruses. The wood density decreased only by about 1.15 %, the modulus of elasticity in longitudinal direction measured by the speed of ultrasound waves dropped by about 9.3 %, and the modulus of rupture in bending decreased according to mechanical test by 17 %. In addition, the influence of other species of bacteria for degradation of pits and for increasing the permeability of conifers has been researched. For example, an increase of permeability of pine wood after pre-treatment with the bacterium *Pseudomonas* sp. was observed (Burnes et al. 2000). Suitable bacteria cause a high increase of permeability in sapwood zone of logs and a minimum decrease of mechanical properties because they destroy only pits and cell walls remain unchanged.

MATERIAL AND METHODS

In our previous experiment "combined ponding – bio-treatment with bacteria" the green spruce logs were primarily ponded in non-sterile water during 16 weeks, and then the individual sapwood and heartwood specimens prepared from ponded logs were subsequently exposed to the bacterium *Bacillus subtillis* (Pánek et al. 2005).

Now the green spruce specimens were directly exposed to the bacteria *B. subtilis*. For this work we used a healthy 79 year-old spruce tree from which two types of specimens have been immediately prepared (Fig. 1):

- 30x30x15 mm for the coefficient of axial permeability testing (n = 330),
- 20x20x30 mm for the kinetics of water soaking testing (n = 240).

Medium density of the sapwood specimens was 419.5 kg/m^3, and that of the heartwood specimens was 358.1 kg/m^3.

Bio-treatment of green spruce specimens was carried out in four glass vessels 500x300x250 mm filled with the bacterial culture (*Bacillus subtilis* in the distilled water) using a hydro-module of 1:5. All surfaces of these specimens were first sterilized with UV radiation. Bacterial incubation of specimens at a temperature of 20 ± 2 °C or 30 ± 2 °C lasted 1, 3, 6 or 9 weeks (Fig. 1). The bacterial culture was put into glass vessels either only at the start of experiment – into two vessels, or it was additionally charged each week – into another two vessels (Fig. 2). Simultaneously, other sapwood and heartwood specimens were ponded for 4 months in non-sterile water.

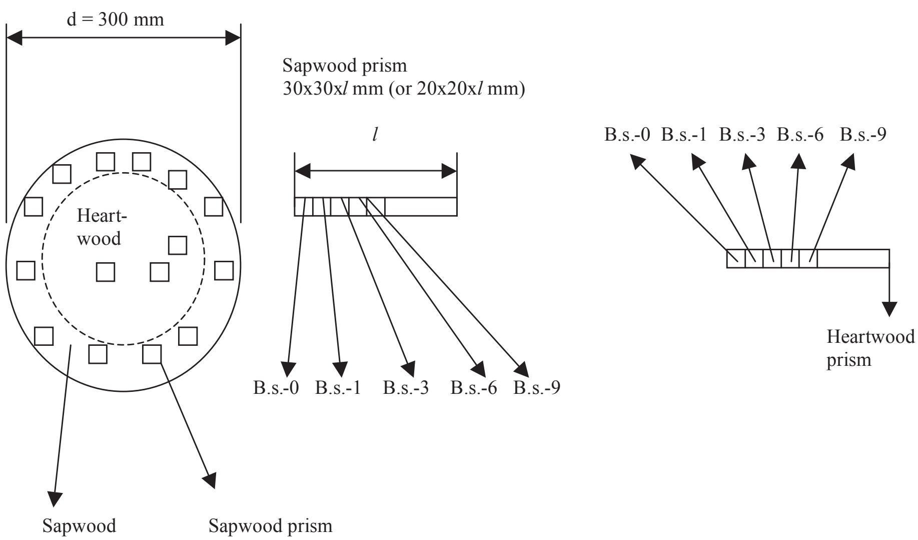


Fig. 1: Scheme of samples preparation from the green spruce log
(Note: B.s.-1 = Sample exposed to the bacterium B. subtilis during 1 week)

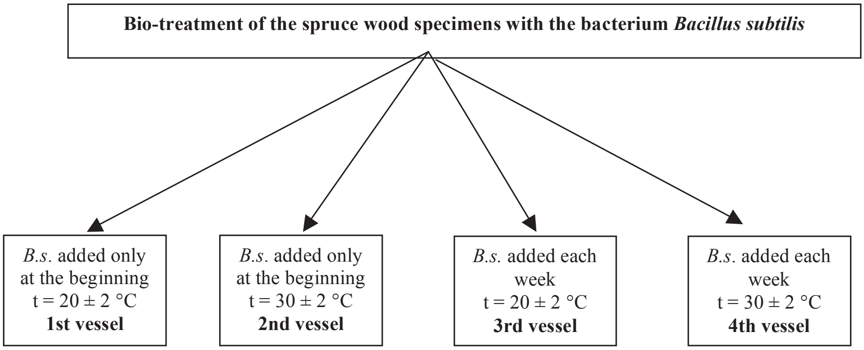


Fig. 2: Scheme of the experiment with Bacillus subtilis

Spruce specimens:
- control,
- bio-attacked by *Bacillus subtilis,*
- ponded for 4-months in non-sterile water,

have been before testing of their structural changes and physical properties first sterilized for 5 hours at 90 °C. Specimens designed for the determination of the coefficient of permeability were then conditioned to a moisture content of 12 %, and those designed for the determination of the kinetics of water soaking were dried to a moisture content of 0 %. Selected specimens were submitted also to microscopic analyses with the aim to find changes in the pit membranes or other changes in cell walls.

The coefficient of permeability in axial direction (L = 15 mm) was determined on the basis of the Darcy low:

$$K = (V \,.\, \eta \,.\, L) : (A \,.\, \tau \,.\, \Delta p) \; (m^2)$$

where: V – transported volume of distilled water (m^3), η – dynamic viscosity of distilled water at 20 °C (Pa.s), L – specimen length = 0.015 m, A – axial surface allocated for the flow transport (m^2), τ - time of flow (s), Δp – pressure difference (Pa).

The kinetics of soaking in distilled water was determined in the interval from 15 minutes to 21 days in these time-intervals: 15 min, 30 min, 45 min, 1 h, 2 h, 4 h, 8 h, 1 day, 2 days, 3 days, 6 days, 8 days, 10 days, 16 days, and 21 days.

RESULTS AND DISCUSSION

Results of this work are presented in the Tab. 1, Fig. 3-6, and Photo 1.

The bacterium *Bacillus subtilis* was able to open pit membranes only in the sap zones of spruce specimens (Photos 1B-1D) which resulted in their apparently increased permeability (Tab. 1 and Fig. 3) and kinetics of water soaking (Fig. 4 and 5). The total degradation of sapwood pit membranes, and a subsequent expressive increase of sapwood permeability and kinetics of soaking was observed already after 1 week of spruce bio-treatment at the optimum temperature of 30 °C for the bacterium *B. subtilis*. At a lower temperature of 20 °C the similar results have been achieved only at longer times of bio-treatment processes lasting 3-9 weeks. The coefficients of permeability of such bio-treated sapwood specimens increased approximately from 7 to 24.6 times, and were even higher than those of the green sapwood (Tab. 1). However, the non-optimal bio-treatments with *B. subtilis* at 20 °C lasting only 1 week had a minimum positive effect on sapwood impregnability (Tab. 1, Fig. 3-5). After 4 months of ponding the permeability of sapwood specimens increased by 6.6 times (Tab. 1).

Tab. 1: The coefficients of axial permeability (K) of spruce sapwood in the natural state (green wet state w >> 30 %), in untreated state – control (dried at w = 12 %), after bio-treatment with the bacterium Bacillus subtilis during 1, 3, 6 or 9 weeks (dried at w = 12 %), and after ponding for 4 months in non-sterile water (dried at w = 12 %)

COEFFICIENT OF PERMEABILITY OF SPRUCE SAPWOOD								
Kind and time of bio-treatment	Methods of bio-treatment with the bacterial culture *Bacillus subtilis*							
	1st method 20 °C / added at beginning		2nd method 30°C / added at beginning		3rd method 20 °C / added each week		4th method 30 °C / added each week	
	$K.10^{12}$ (m^2)	v (%)	$K.10^{12}$ (m^2)	v (%)	$K.10^{12}$ (m^2)	v (%)	$K.10^{12}$ (m^2)	v (%)
0 week (Control)	0.37	33.,6	0.42	36.3	0.49	42.3	0.37	26.3
1 week	0.79	70.2	6.46	24.7	0.62	24.6	9.11	43.6
3 weeks	7.04	15.6	6.48	23.1	3.45	61.0	5.15	28.7
6 weeks	8.03	16.5	7.72	17.0	7.22	16.8	6.85	17.8
9 weeks	5.78	14.4	7.48	12.5	7.34	9.6	6.48	28.5
Green wet state	$K = 4.30.10^{-12}$ m^2 (v = 35.8 %)							
4 month-ponding	$K = 2.71.10^{-12}$ m^2 (v = 38.2 %)							

Note: v – coefficient of variability, n – number of specimens at each group = 15

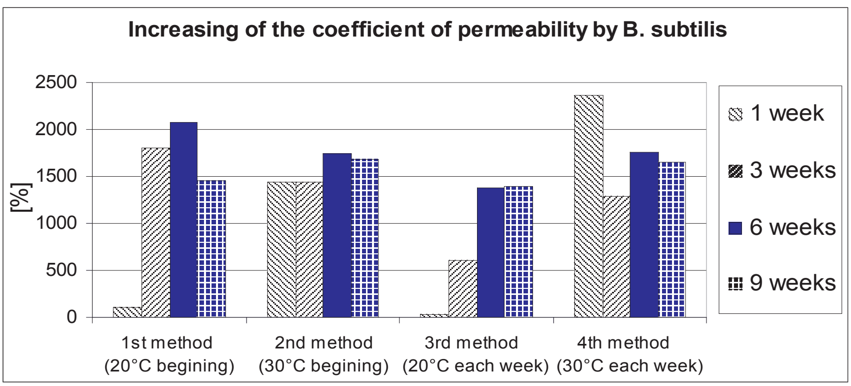


Fig. 3: Percentage increase of the coefficients of axial permeability (K) of spruce sapwood treated with the bacterium Bacillus subtilis using four treatment methods lasting 1, 3, 6 or 9 weeks
Note: The permeability was measured at dry state of spruce specimens (w = approximately 12 %)

1st method (20 °C)

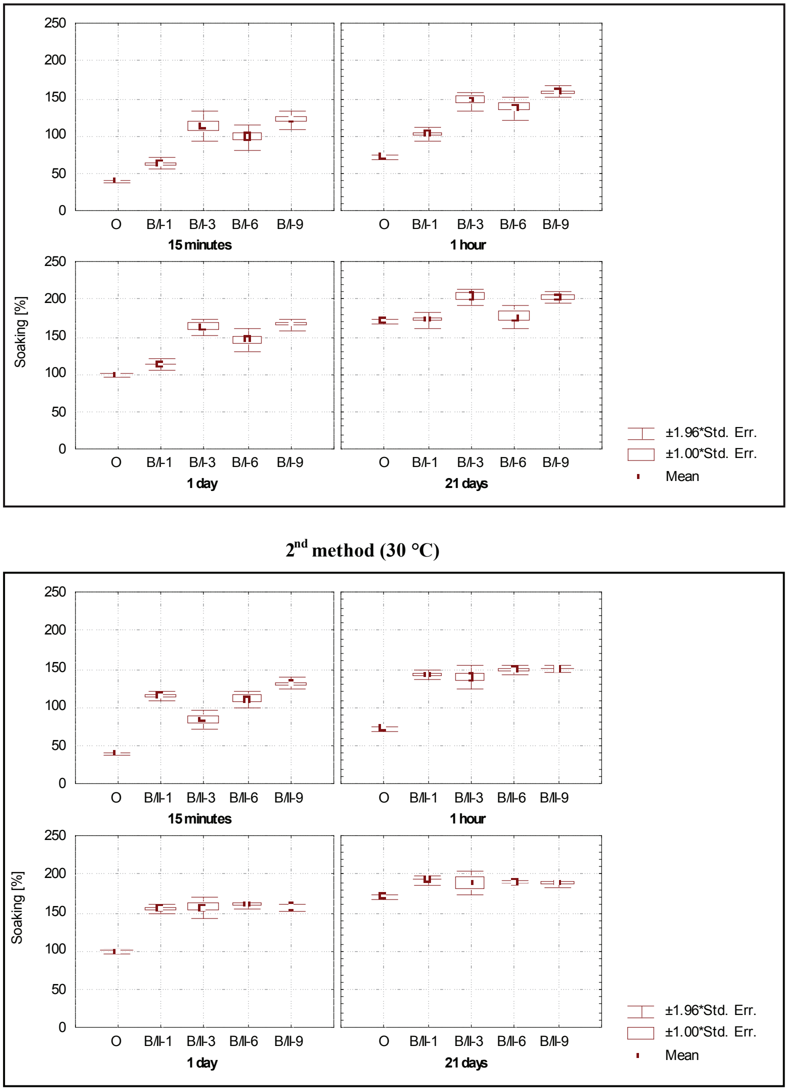


Fig. 4: Two side confidence intervals of water soaking of untreated spruce sapwood (O) and spruce sapwood bio-treated for 1, 3, 6 or 9 weeks with the bacterium Bacillus subtilis (B/1, B/3, B/6 and B/9) using the 1st method at 20 °C (I.) or the 2nd method at 30 °C (II.) – soaking in distilled water measured after 15 minutes, 1 hour, 1 day and 21 days

Notes for Fig. 4:

B/I. – 1st bio-treatment method (B. subtilis added at the beginning, t = 20 °C)

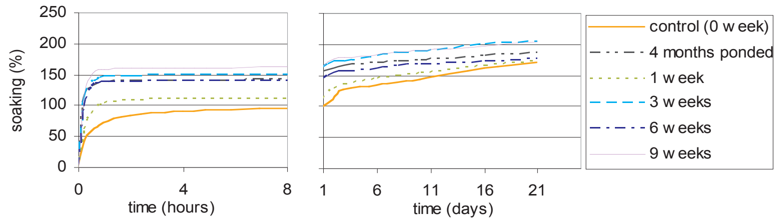
B/II. – 2nd bio-treatment method (B. subtilis added at the beginning, t = 30 °C)

1, 3, 6 or 9 – time of the bio-treatment in weeks

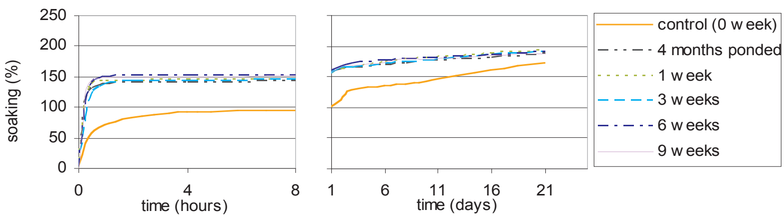
n – number of specimens at each group = 5

SPRUCE SAPWOOD

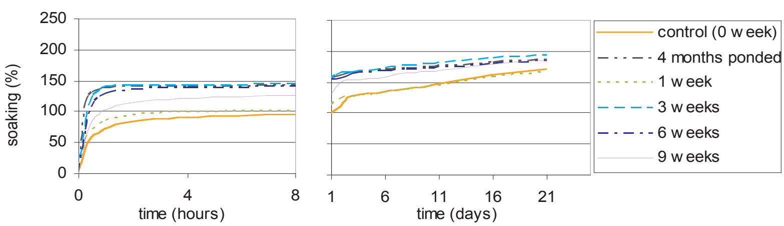
Bacillus subtilis added at the beginning (t = 20°C)



Bacillus subtilis added at the beginning (t = 30°C)



Bacillus subtilis added each week (t = 20°C)



Bacillus subtilis added each week (t = 30°C)

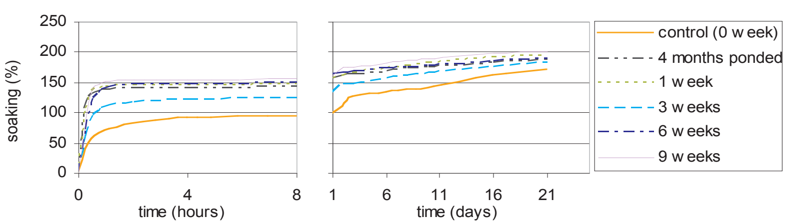


Fig. 5: The kinetics of water soaking of spruce sapwood specimens bio-treated with Bacillus subtilis (1, 3, 6 or 9 weeks) in comparison with control specimens and those ponded for 4 months in non-sterile water
Note: Soaking was measured in these times: 15 min, 30 min, 45 min, 1 h, 2 h, 4 h, 8 h, 1 day, 2 days, 3 days, 6 days, 8 days, 10 days, 16 days, 21 days.
n – number of specimens at each group (for each curve line) = 5

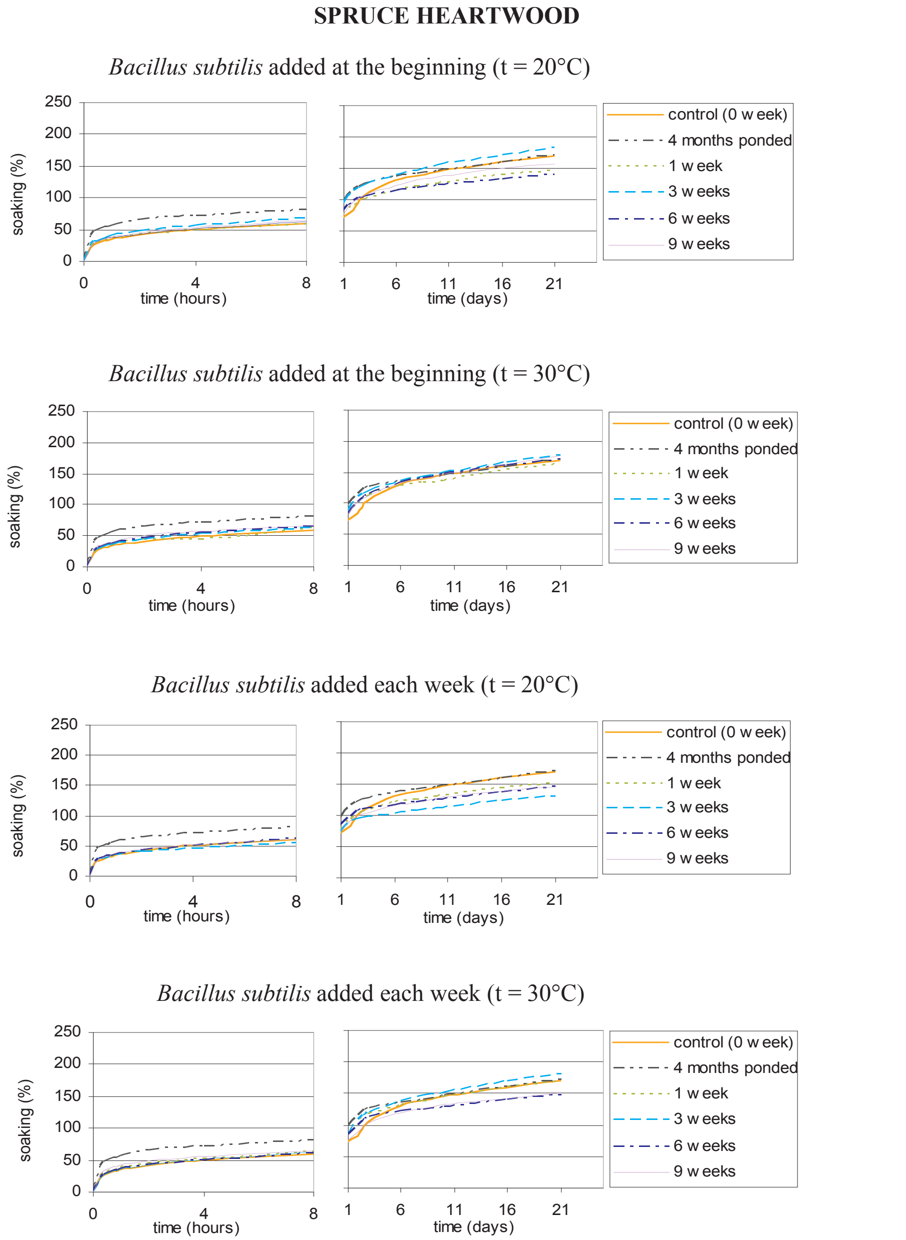


Fig. 6: The kinetics of water soaking of spruce heartwood specimens bio-treated with Bacillus subtilis (1, 3, 6 or 9 weeks) in comparison with control specimens and those ponded for 4 months in non-sterile water
Note: Soaking was measured in these times: 15 min, 30 min, 45 min, 1 h, 2 h, 4 h, 8 h, 1 day, 2 days, 3 days, 6 days, 8 days, 10 days, 16 days, 21 days.
n – number of specimens at each group (for each curve line) = 5

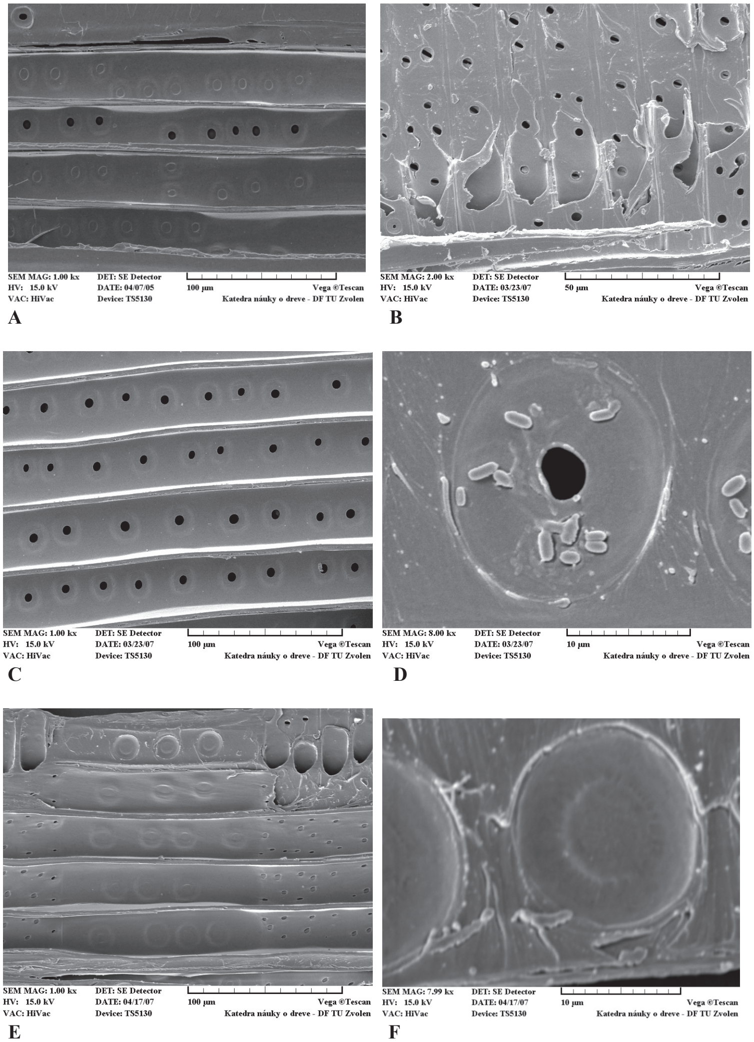


Fig. 7: Changes in the structure of spruce wood after bio-treatment with the bacterium Bacillus subtilis

A – pits in the tracheids of untreated spruce sapwood were obviously closed, B – strong destruction of membranes of parenchyma cells of sapwood bio-treated with the 2nd method (30 °C / bacteria added at the beginning) during 9 weeks, C – tracheids of sapwood bio-treated with the 2nd method (30 °C / bacteria added at the beginning) during 9 weeks, D – detail on the opened pit membrane of sapwood and individual bacteria after bio-treatment with the 2nd method (30 °C / bacteria added at the beginning) during 1 week, E – pits in tracheids of spruce heartwood remained closed after bio-treatment with the 4th method (30 °C / bacteria added each week) during 3 weeks, F – closed pit of the tracheid of spruce heartwood bio-treated with the 4th method (30 °C / bacteria added each week) during 3 weeks.

When measuring the kinetics of water soaking, in the first minutes and hours we observed a high increase of water uptake into the bio-treated sapwood spruce specimens. The highest increase was observed during the first 15 minutes, maximally to 227 %. In this time, the maximum values of soaking were observed already after 1 week of bio-treatment at 30 °C, or after 3-9 weeks at 20 °C (Fig. 4 and 5). This result is similar to permeability (Tab. 1, Fig. 3). With soaking time prolongation, the differences of water uptake between untreated and bio-treated sapwood specimens were smaller. For example, after 1 hour of soaking the increase was maximum to 123 %, at which after 21 days of soaking there already were not any more evident differences (Fig. 4 and 5). Kinetics of water soaking of the sapwood specimens ponded for 4 months was similar to those which were sufficiently bio-treated with *B. subtilis*.

On the other hand, the bio-treatments with *B. subtilis* did not have positive effect on the opening of pit membranes, permeability and kinetics of water soaking of heartwood spruce specimens (Fig. 6, Fig. 7E and 7F). Small differences in soaking of the untreated (control) and bio-treated heartwood spruce specimens can be explained only on the basis of differences in their densities. Toruses in the pits of heartwood are permanently closed because they contain some material similar to lignin (Côte 1963), at which the bacterium *B. subtilis* does not create enzymes for lignin and similar polyphenol compounds decomposition (Eriksson et al. 1990). However, a minimum positive effect on the kinetics of water soaking of spruce heartwood specimens (mainly in the initial periods) was observed after ponding them for 4 months in non-sterile water.

Our results coincide well with those of some other authors working in this field. For example, Efransjah et al. (1989) observed an increase in permeability of sapwood zone of spruce wood after 5 months of ponding with bacterium *Bacillus subtilis.* Similarly Ellwood and Eckland (1959), Dunleavy and McQuire (1970), Unligil (1972), Singh et al. (1998) observed an increase in permeability of conifers after their ponding in non-sterile water resulting in destroying of pit membranes by various bacteria. All these results have shown that bio-treatment of coniferous wood species is also suitable for technical applications – e.g. for improving the impregnability of spruce, fir and pine poles.

CONCLUSIONS

The bio-treatment of spruce sapwood with the bacterium *Bacillus subtilis* had a sufficient effect on improving its impregnability already after 1 week of exposure at an optimal temperature of 30 °C for the bacteria growth. It was documented by opened pit membranes (Fig. 7B, 7C and 7D), increasing of the coefficient of permeability in axial direction and the kinetics of water soaking (Tab. 1, Fig. 3 - 5). However, the bio-attack of sapwood spruce with *B. subtilis* at a lower temperature of 20 °C had a sufficient effect on the increase of permeability and speed of soaking only after a longer time lasting 3-9 weeks. We observed that the coefficient of permeability of the adequately bio-treated spruce sapwood specimens obviously increased about 1500-2000 % in comparison with untreated ones, and their kinetics of soaking increased expressively mainly in the first minutes of the test by about 120-220 %.

On the other hand, the structure of spruce heartwood was not changed by the bacterium *Bacillus subtilis*. It was documented by the microscopic analyses and measuring of kinetics of water soaking (Fig. 6, Fig. 7E and 7F). Speed of soaking in the initial periods was the same for both the bio-treated and untreated heartwood specimens.

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