BACILLUS SUBTILIS FOR IMPROVING SPRUCE WOOD IMPREGNABILITY

Miloš Pánek and Ladislav Reinprecht

Impregnation of spruce and other conifers is very difficult due to aspiration of pits in tracheids. In this experiment, freshly cut and instantly debarked Norway spruce logs were pre-treated with the bacterium *Bacillus subtilis* at their ponding for 1, 3, 6 and 9 weeks under laboratory conditions at 30 °C or under outdoor conditions during the summer at 5 to 35 °C. Significant increases of the permeability and the impregnability of spruce sapwood were observed already after 3 weeks of its ponding due to bacterial attack and opening of pits in tracheids. Applied bio-treatments did not have a significant influence on selected mechanical properties of spruce wood. This method could be effectively used for poles and other round products from spruce or other conifers before their impregnation with preservatives or modification substances. However, its use for squared spruce timbers is not convenient because tracheids in the bacterially treated heartwood zone remained unchanged.

Key words: Impregnability; Spruce; Biological treatment; Bacteria; Bacillus subtilis

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INTRODUCTION

Transport processes of liquids in wood cells, and also in wooden products depends on many factors, including the structure of the wood, its moisture content, properties of the liquid, and pressure and diffusion gradients (Kurjatko and Reinprecht 1993). The microscopic structure of hardwoods and softwoods is not simple, and transport processes in cells and among cells of wood are often very complicated. Various mathematical models of liquid transport in wood have been carried out (e.g. Bolton and Petty 1978; Comstock 1963, 1970; Reinprecht 1993; Ronze et al. 1988; Siau 1984, 1995; Siau and Babiak 1983; Skaar and Babiak 1982). The influence of wood structure, as for its permeability and impregnability, has been summarised in the works of Siau (1984, 1995), Hansmann et al. (2002), Usta (2005), Lehringer et al. (2009), and others.

Impregnability of spruce wood, as well as other coniferous trees, is very difficult due to aspiration of pits in tracheids during the tree growth (in heartwood) or during the drying process after cutting the fresh trees (in sapwood) (Mamoňová 1999, 2000). This problem has not been optimally solved until now. In practice various kinds of mechanical or laser incising and cutting technologies are usually used (Goodel et al. 1991; Morrell and Morris 2002; Reinprecht and Horský 1990; Smith et al. 1990; Smith and Morrell 1991) with the aim to increase the frontal surfaces of wood.

There are also possibilities of physical pre-treatments of conifers, such as using ultra-sound waves during impregnation (Marčok and Kurjatko 1988), or using special methods of seasoning (Booker and Evans 1994; Moldrup 1995; Morrell and Morris 2002; Morris et al. 1998). Militz and Homan (1993) analysed various chemical pre-treatments of conifers for improving their impregnability. Another useful way for pits destruction is the autocatalytic effect of acetic acid during the boiling or steaming of wood (Nicholas and Thomas 1968).

Biological pre-treatments of conifers using bacteria, enzymes, or fungi have been investigated from the middle of the 20th century by various researches, including Ellwood and Eckland (1959), Liese and Greaves (1975), Militz (1993), and Messner et al. (2003). Also in our previous works, the influence of the bacterium *Bacillus subtilis* and the microscopic fungus *Trichoderma viride* for increasing the impregnability of spruce wood was tested in laboratory conditions on small samples (Pánek et al. 2005; Pánek and Reinprecht 2008; Reinprecht and Pánek 2008).

Generally, two main bio-treatment methods have been tested: Ponding, or spraying of logs in non-sterile conditions with parallel activity of more species of bacteria – mainly in natural lakes or basins (Banks 1970; Despot 1993; Dunleavy and McQuire 1970; Ellwood and Eckland 1959; Singh et al. 1998; Unligil 1972; and others). The necessary time for pre-treatment of logs was determined from 3 to 6 weeks.

Use of specific pure cultures of bacteria and observation of their influence on wood structure, has mainly focused on increasing of its permeability (Burnes et al. 2000, Efransjah et al. 1989, Highley and Lutz 1970, Schmidt et al. 1987, and others). Preferentially the bacteria species used have been those from the genus of *Bacillus* and *Pseudomonas,* but also from the genus of *Clostridium* and *Cellulomonas*.

Effects of bacterial attack to wood were summarised in the works of Greaves (1969, 1971), Liese and Greaves (1975), Eriksson et al. (1990), and Schmidt and Liese (1994). Greaves (1971) divided the effect of bacteria to wood into four main groups:

1. Bacteria degraded cell contents in lumina of parenchyma, and also the pits in cell walls; however without a more evident effect on the mechanical properties of wood.
2. Bacteria that degrade the cell walls of wood.
3. Bacteria whose activity on wood is connected with parallel or previous degradation of wood by other biotic agents, or by previous atmospheric corrosion of wood.
4. Antagonistic bacteria which protect wood against other biodegradation agents, e.g. against wood-destroying fungi.

Bacteria from the first group are the most suitable for focused pre-treatments of wood. They do not produce the complicated complex of enzymes necessary for degradation of cell walls in wood. Bacteria from the second group (possible attack of wood during non-sterile ponding) cause cell wall degradation very slowly and the decrease of the strength of wood is relatively small even after a long period of ponding (Adolf et al. 1972; Efransjah et al. 1989).

In practice, the applicability of bacteria for increasing the impregnability of refractory conifers should be easier and more effective in comparison to the use of microscopic fungi. The main advantage of bacteria is their possibility to create more

homogenous dispersions convenient for pre-treatment technologies, and also their very simple application. The ponding technology appears to be more suitable than the spraying one, because with spraying of bacteria it is more difficult to achieve their uniform attack to all surfaces of logs. The parallel protection function of ponding in non-sterile water is connected with a physical protection of wood (its high moisture content without air needed for growth of fungi) and also with an antagonistic effect of some bacteria species (including the *Bacillus subtilis*) against wood-destroying fungi and moulds (Moita et al. 2005; Pánek and Vidholdová 2009; Tiralová et al. 2007).

The aim of this work was to achieve a deeper knowledge about the influence of the bacterium *Bacillus subtilis* on the impregnability of hardly permeable tree species, and on their selected mechanical properties. Experiments were focused especially to observe influence of different environmental conditions (temperature, time) and concentration of bacterium for permeability increasing of spruce sapwood and heartwood, respectively. Potential structural changes of bio-treated wood have been evaluated on the base of the coefficient of permeability and the retention values at soaking and pressure impregnation processes. Impact bending strength and bending strength have been researched in detail as the most sensitive mechanical properties of bio-damaged woods.

Selection of the bacterium *B. subtilis* for this experiment was based on several reasons: this bacterium is common in soil, water and also in the human organism; it is non-toxic for people and also for aquatic organisms, meaning that it is environmentally friendly; it is a highly resistant, so under unfavorable conditions it creates and waits in the state of endospores; and it increases durability of wood against other wood-destroying agencies. Using of this bacterium is a cheap and relatively simple method for increasing of impregnability of round refractory wood products, and by our opinion worthy of attention.

EXPERIMENTAL

Materials

In our previous works, the effect of the bacterium *Bacillus subtilis* for improving impregnability of the Norway spruce was analyzed by the method of bio-treatment of small sapwood and heartwood samples (Pánek et al. 2005; Pánek and Reinprecht 2008). In this work, the pure culture of the bacterium *B. subtilis* CCM-1 was applied for the bio-treatment of freshly cut and debarked spruce logs.

Spruce wood

Norway spruce (*Picea abies* Karst. L.) bolts, i.e. short logs with a medium diameter from 230 to 250 mm, and with a constant length of 600 mm, were used in the experiment. The median density of the sapwood was 395.1 kg/m^3, and that of the heartwood 345.3 kg/m^3, respectively. Surfaces of all 14 bolts intended for bio-treatments, and also of 1 control bolt (O-untreated), were firstly sterilized by UV-radiation with 800 W during 30 minutes. Frontal surfaces of bolts were then painted with PVAc latex for

similarity of their artificial bacterial pre-treatment with practical ponding of several meters-long logs.

It is known that median number of pits in the tracheids of Norway spruce logs growing in Slovakia is from 70 to 90 in the earlywood, and from 8 to 25 in the late wood (Požgaj et al. 1993). Median area porosity of the Norway spruce wood is from 37 to 40% for heartwood, and from 44 to 46% for sapwood (Masaryková and Čunderlík 2011).

Bacterium Bacillus subtilis

A water suspension of the bacterium *B. subtilis* CCM-1 was used in the experiment.

Bacterial Pre-treatment of Spruce Bolts - Short Logs

The bio-treatment of spruce bolts was carried out in plastic vessels 300 x 450 x 700 mm by their ponding in water suspension of the bacterium *Bacillus subtilis* CCM-1 at the hydromodule of 1:3. Bacteria were added into each plastic vessel from 10 Petri dishes ∅ 120 mm in a suspension with distilled water. Ponding of bolts in bacterial suspension at sterile conditions lasted 1, 3, 6, or 9 weeks. The following types of bacterial pre-treatments of spruce bolts have been used:

B I – bacteria added at the beginning of ponding; attack in a laboratory at 30 °C,
B II – bacteria added each week of ponding; attack in a laboratory at 30 °C,
B III – bacteria added at the beginning of ponding; attack in exterior conditions during summer at temperatures ranging from 5 to 35 °C,
B IV – bacteria added each week of ponding; attack in exterior conditions during summer at temperatures ranging from 5 to 35 °C.

These bacterial pre-treatments B I, B II, B III, and B IV, entailed different selected concentrations of the bacteria (added at beginning or each week), and also different environmental conditions (laboratory or exterior).

Permeability, Impregnability, Strength, and Microscopic Analyses of Samples from Bacterially Pre-Treated Spruce Bolts

From the bacterially pre-treated bolts, and also from the control bolt (O-untreated), testing samples were immediately prepared (Fig. 1).

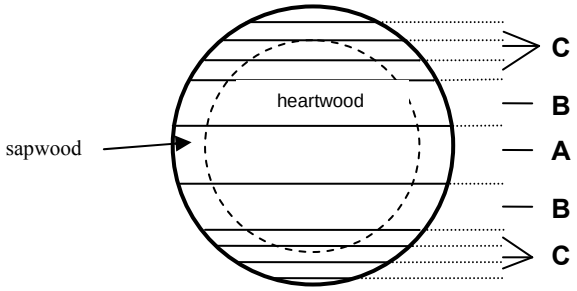


Fig. 1. Scheme of testing samples preparation from one bolt with the length of 600 mm. **A** zone – samples for the coefficient of permeability (n=15); **B** zone – samples for the water uptake (n=5) and for the impregnability (n=5); **C** zone – samples for the modulus of rupture (n=10) and for the impact bending strength (n=10)

All testing samples from the sapwood and heartwood zones were sterilized for 5 hours at 90 °C, then conditioned at two moisture content levels of w = 0 % or 8±1 %, and finally used for the these analyses:

Coefficient of axial permeability (K)

The coefficient of axial permeability was determined on the basis of the Darcy´s law, using steady flow of 20 °C distilled water through wood samples in the apparatus by Reginác et al. (1977) and the methodology by Pánek and Reinprecht (2008).

Experimental conditions were as follows: L - length of samples in the longitudinal "axial" direction (0.015 m); A - inputting transversal area of samples for flow (1.10^{-4} m^2); Δp - pressure difference (1.10^5 Pa); V - transported volume of distilled water (5.10^{-6} m^3); η - dynamic viscosity of distilled water at 20 °C (1.10^{-3} Pa.s); τ - time of flow (s); initial moisture of samples w = 8±1 %; and number of samples in each series n = 15.

$$K = (V \,.\, \eta \,.\, L) : (A \,.\, \tau \,.\, \Delta p) \qquad [\mathrm{m}^2] \tag{1}$$

Water uptake – kinetics of water soaking (S)

Soaking of samples 20 x 20 x 30 mm /RxTxL/ in distilled water at 20 °C was evaluated after 15, 30, and 45 minutes, 1, 2, 4, and 8 hours, and 1, 2, 3, 6, 8, 10, 16, and 21 days, by Equation 2.

$$S = /(m_w - m_0) : m_0/ \,.\, 100 \qquad [\%] \tag{2}$$

where: m_0 - weight of sample in the oven dry state (dried at 103±2 °C); m_w – weight of sample in a defined stage of soaking. In each series 5 samples were tested.

Impregnability (R, I_S)

Impregnability was determined on the basis of the retention of distilled water into samples at modified Lowry impregnation technique (R in kg/m^3), and also on the basis of the degree of their saturation with distilled water (I_S in % by Equation 3),

$$I_S = (R \,/\, R_{max}) \,.\, 100 \qquad [\%] \tag{3}$$

where R is the achieved retention of water in the sample and R_{max} - is the theoretically maximum retention of water in the sample with a defined density (porosity) and moisture.

Experimental conditions were as follows: RxTxL are the dimensions of samples (20x20x100 mm); Δp is the pressure difference at beginning of each impregnation cycle (8.10^5 Pa); τ is the time of pressure impregnation (5, 15 and 150 minutes); frontal surfaces of samples were painted before impregnation by paraffin; initial moisture of samples w = 8±1 %; and number of samples in each series n = 5;

Modulus of rupture (MOR) and impact bending strength (IB)

MOR and IB were determined by the ASTM D143-94 (2000) e1 standard, using modified dimensions of samples.

Experimental conditions were as follows: *R*x*T*x*L* were the dimensions of samples (10x10x120 mm); the moisture of samples *w* were 8±1 %; and the number of samples in each series $n = 10$.

Microscopic analyses

These were done with an electron microscope of type RAM-TESCAN-VEGA TS 5130.

Statistical Evaluation

All the results achieved for the bio-treated spruce sapwood and heartwood samples were compared with those of the biologically untreated ones (O-untreated). The results were statistically evaluated by mean values, line plots, box and whiskers plots, standard deviations, and statistically analyzed by the Duncan test.

RESULTS AND DISCUSSION

Coefficient of Permeability and Water Uptake

Changes in the permeability of the bio-treated spruce sapwood and heartwood were detected, first of all, by changes in the coefficient of axial permeability, for which a constant liquid pressure difference of 0.1 MPa/15 mm has maintained (Fig. 2), and then by changes in the water uptake at atmospheric pressure (Figs. 3 and 4).

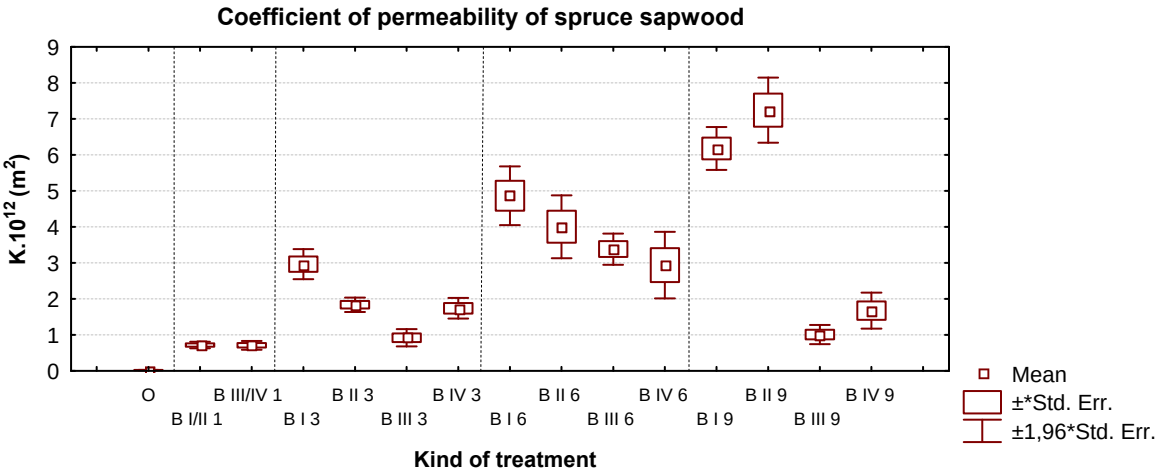
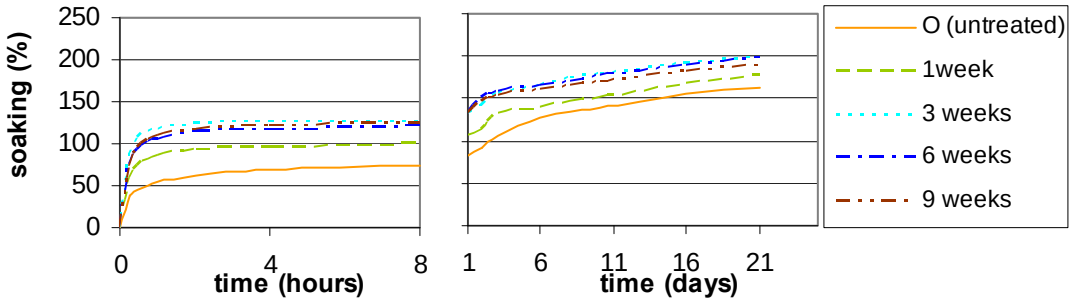
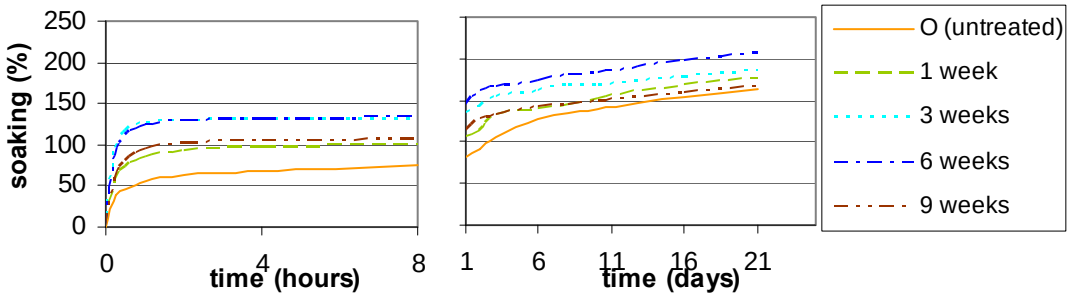


Fig. 2. Coefficient of axial permeability (K) of spruce sapwood from bolts bio-treated with the bacterium *B. subtilis*

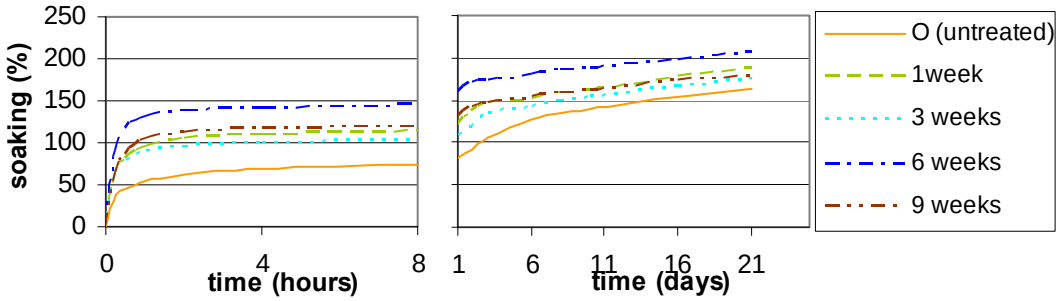
The coefficient of axial permeability (K) of the bio-treated spruce increased significantly in the case of sapwood (Fig. 2). On the other hand, this coefficient could not be determined for the spruce heartwood before and after bio-treatment. In our experiment, after 1 week of bio-treatment the K value of spruce sapwood increased approximately 5 times in comparison to untreated sapwood. After 3 weeks of bio-treatment it increased from 6.7 times (B III 3 – type of treatment) to 24 times (B I 3 – type of treatment).



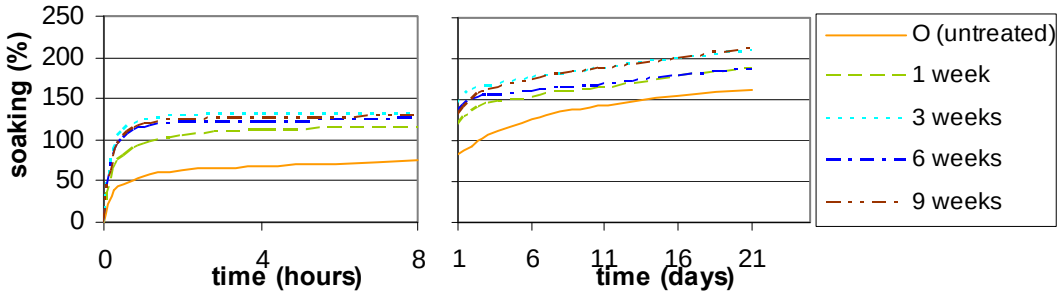
a) Bacteria added at beginning in a laboratory (B I)



b) Bacteria added each week in a laboratory (B II)



c) Bacteria added at beginning in exterior conditions (B III)



d) Bacteria added each week in exterior conditions (B IV)

Fig. 3. Water soaking (S) of spruce sapwood from bolts bio-treated with the bacteria *B. subtilis*

The maximum improvement, a 59-fold increase of the coefficient of permeability of spruce sapwood (on the value of K = $7.24.10^{-12}$ m^2), was observed after 9 weeks of the *B. subtilis* action in the laboratory – when the bacteria suspension was added to spruce logs each week (B II 9; see Fig. 2). Prolongation of the bacterial attack from 1 to 9 weeks evidently had a positive effect only under the laboratory conditions (Fig. 2). This result was obviously caused by a higher temperature in the laboratory, because the *B. subtilis* exhibits optimal growth at temperatures near 30 °C, and at lower temperatures its activity is a smaller (Pánek and Reinprecht 2008). In addition, exterior environmental conditions could not be so controlled, such that biological processes in spruce pits were probably more variable. An increase of the permeability of spruce sapwood after ponding of logs in the presence of *B. subtilis* was observed also Efransjah et al. (1989).

The water uptake (S), measured from 15 minutes to 21 days, increased only for the bio-treated spruce sapwood (Fig. 3); however the effect was less pronounced in comparison to its axial permeability. The water uptake increased significantly in the initial stages of the soaking test from 0 minutes to 24 hours (1 day), but above all in its first 15 minutes. After 1 week of bacterial attacks, the soaking S_{15min} increased by 52 % (B I 1, and B II 1 → bio-treatments in a laboratory), or by 69 % (B III 1, and B IV 1 → bio-treatments in an exterior). The effect of the bacterium *Bacillus subtilis* was more pronounced at longer times of bio-treatments; e.g. after 3 weeks of bacterial attacks, the soaking S_{15min} increased already by 129 to 150 % (B I 3, and B II 3), or by 75 to 136 % (B III 3, and B IV 3; see Fig. 3).The water uptake (S) of the bio-treated spruce heartwood remained practically unchanged (Fig. 4). This result corresponds with previous works of other researchers, e.g. of Unligil (1972); Schmidt et al. (1987); or Despot (1993).

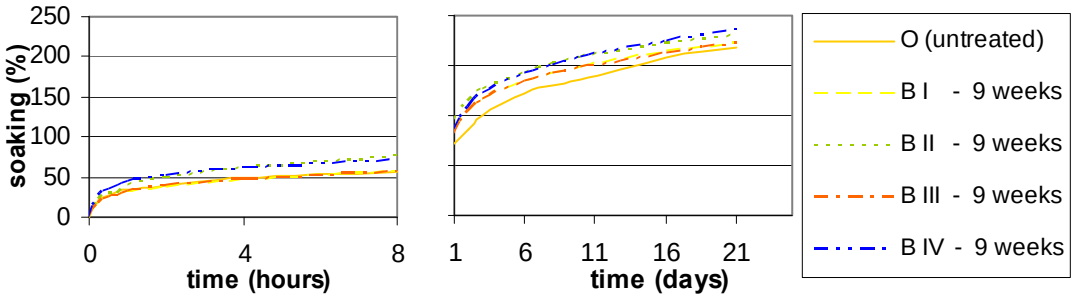


Fig. 4. Water soaking (S) of spruce heartwood from bolts attacked with the bacteria *B. subtillis* during 9 weeks in laboratory (B I; B II) or in exterior (B III; B IV)

Impregnability

The impregnability of wood is an important characteristic with respect to its suitability for chemical preservations and modifications by pressure techniques. This characteristic, similar to the coefficient of permeability and the water uptake, is also dependent on the microscopic structure of cell elements of wood, e.g. with their orientation, dimension, and opening of pits. Increases of the retention (*R*) and the degree of saturation (I_S) of the bio-treated spruce sapwood samples in comparison to untreated ones were very similar. In the paper, the I_S values (Tab. 1) and their changes with prolongation of the impregnation process (Fig. 6) are presented for all the samples, while

the *R* values are mentioned only for the samples that had been subjected to 5 minutes of the impregnation process (Figs. 5 and 7).

The impregnability of the bio-treated spruce sapwood increased mainly at its shortest 5 minute time of pressure impregnation (Tab. 1, Figs. 5 and 6).

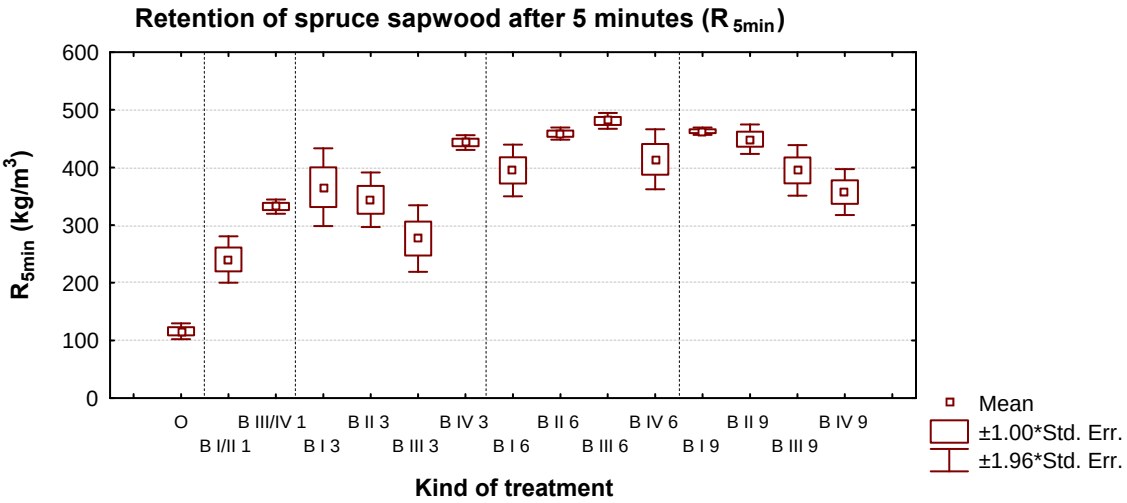


Fig. 5. Impregnability – retention after 5 minutes at pressure impregnation (R_{5min}) of spruce sapwood from bolts bio-treated with the bacteria *B. subtilis*

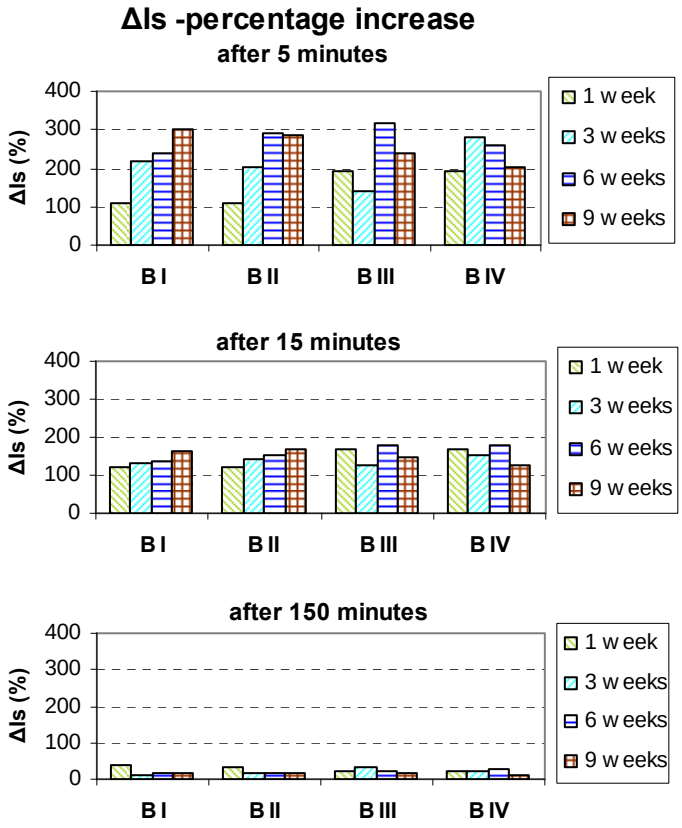


Fig 6. Increase of the degree of saturation $\Delta I_S = [(I_S - I_{S\text{-untreated}}) : I_{S\text{-untreated}}] \cdot 100$ (%) after 5, 15, and 150 min of pressure impregnation of the spruce sapwood pre-treated with *B. subtilis*

For example, after 1 week of bio-treatments with *Bacilus subtilis* the retention of spruce sapwood at the beginning of impregnation (R_{5min}) increased by 107% as a result of the action of bacteria in a laboratory (B I, B II), and by 187 % at their action under exterior conditions (B III, B IV). The tendency of increasing the degree of saturation ($I_{S\text{-}5min}$) was similar, by 110% (B I, B II) and 193% (B III, B IV). After 3, 6, and 9 weeks of bio-treatments, the R_{5min} increased by 138 to 314%, and the $I_{S\text{-}5min}$ was higher by 141 to 318%. A similar increase in the impregnability of spruce sapwood by about 155% was also determined by Unligil (1972) after ponding of logs in non-sterile water.

The impregnability of spruce sapwood samples bio-treated outdoors for 9 weeks (B III and B IV) was a slightly lower than those bio-treated only 6 weeks (however, it was not observed in the laboratory conditions - B I and B II). This result can be explained by the following two assumptions: (1) exterior environmental conditions are not always easily controlled, so they are more similar to working conditions in practice; and (2) bio-treatments as biological processes can have considerable variability (see Tab. 1).

Differences in the impregnability of the bio-treated and controlled (O-untreated) spruce sapwood samples were evidently lower after 15 minutes and definitely after 150 minutes of impregnation (Fig. 6), in comparison to after 5 minutes; so an even lower number of opened pits in untreated samples the spruce sapwood can be saturated by impregnation solution (distilled water) after longer periods of pressure impregnation. However, after 15 or 150 minutes of impregnation the differences in the impregnability of the bio-treated and untreated samples were still significant (Tabs. 1 and 3).

Finally, it can be said that the bacterial pre-treatments should be important for shortening the time of the sapwood impregnation in spruce and other refractory species when predetermined values of impregnability (I_S or R), and also the depth of penetration have to be achieved. However, the maximum values of impregnability (R_{max}) cannot be significantly increased by this method, because they depend mainly on the porosity of wood.

The impregnability of the bio-treated spruce heartwood was not significantly changed (Fig. 7).

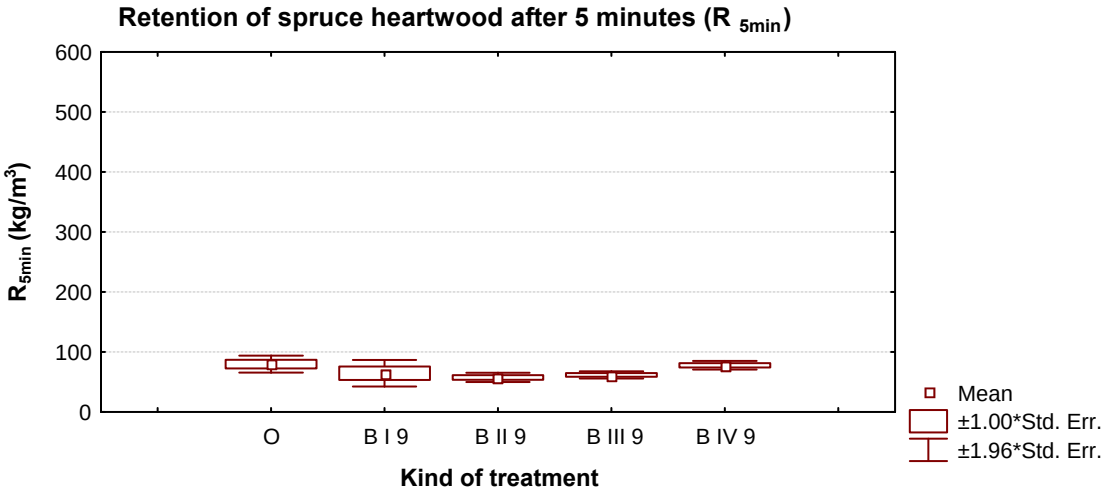


Fig. 7. Impregnability – retention after 5 minutes at pressure impregnation (R_{5min}) of spruce heartwood from bolts bio-treated with the bacteria *B. subtilis*

Table 1. Impregnability – Degree of Saturation (I_S) after 5, 15, and 150 Minutes of Pressure Impregnation of Spruce Sapwood and Heartwood from Bolts Bio-treated with the Bacteria *B. subtilis*

Bacterial treatment of spruce		$I_{S\text{-}5min}$ (%)	$I_{S\text{-}15min}$ (%)	$I_{S\text{-}150min}$ (%)
Sapwood	(weeks)			
O-untreated***	0	14.3* (3.3)**	22.5* (5.3)**	53.0* (10.1)**
B I	1	30.0 (5.7)	49.6 (5.7)	70.7 (2.8)
	3	45.7 (9.5)	52.7 (6.2)	59.9 (4.1)
	6	48.5 (5.3)	53.7 (3.7)	61.9 (1.2)
	9	57.2 (0.9)	59.1 (0.7)	62.1 (1.2)
B II	1	30.0 (5.7)	49.6 (5.7)	70.7 (2.8)
	3	43.0 (6.7)	54.4 (5.0)	61.4 (2.4)
	6	55.8 (1.4)	56.4 (1.2)	62.7 (1.3)
	9	55.5 (3.7)	60.2 (1.8)	62.5 (4.7)
B III	1	41.9 (1.9)	60.4 (2.4)	64.7 (2.9)
	3	34.5 (8.1)	50.5 (8.9)	69.4 (4.1)
	6	59.7 (1.8)	62.6 (1.8)	63.5 (1.4)
	9	48.8 (6.2)	56.0 (5.8)	60.4 (4.5)
B IV	1	41.9 (1.9)	60.4 (2.4)	64.7 (2.9)
	3	54.1 (2.0)	57.2 (1.9)	63.9 (2.9)
	6	51.6 (7.1)	63.2 (2.8)	66.7 (2.3)
	9	43.6 (5.5)	51.3 (2.7)	59.6 (4.2)
Heartwood	(weeks)			
O-untreated***	0	9.7 (2.9)	14.7 (4.3)	33.0 (7.2)
B I	9	7.8 (3.0)	12.0 (2.7)	27.4 (2.7)
B II	9	7.0 (1.1)	10.6 (1.7)	24.3 (2.6)
B III	9	7.5 (0.8)	11.7 (1.6)	28.1 (4.1)
B IV	9	9.4 (1.0)	15.1 (1.7)	36.9 (4.3)

* Arithmetic means in each series are from five samples (n = 5).
** Numbers in the parentheses are the standard deviations.
*** O – untreated: At sapwood used as reference for each week of bio-treatment; at heartwood used as reference for 9 weeks of bio-treatment.

Mechanical Properties

The influence of bio-treatments on the strength of spruce wood was evaluated on the basis of the modulus of rupture (MOR) and the impact bending strength (IB). These properties are very sensitive to changes in the molecular and anatomical structure of wood, and by these properties it is also possible to detected the initial stages of its destruction.

Only relatively minor effects on mechanical properties could be attributed to the attack of spruce sapwood with *B. subtilis*. MOR and IB slightly decreased or slightly increased (Tab. 2), however without any apparent effect of the time or the type of bio-treatments. These conclusions were confirmed also by statistical evaluation using the Duncan test (Tab. 3).

Results of mechanical property tests were in a good correspondence with microscopic analyses, which showed enzymatic degradation of the pits (outage of toruses) and minimum destruction of the cell wall structure of spruce sapwood tracheids (Photos 1-6). Similarly Knuth (1964), Unligil (1972), and Efransjah et al. (1989) observed only a minimum decrease of the mechanical properties in spruce sapwood after its bacterial attack. For example Efransjah et al. (1989) observed a 17% decrease of the MOR after 5 months ponding of spruce logs with *B. subtilis*.

Table 2. Modulus of Rupture (MOR) and Impact Bending Strength (IB) of Spruce Sapwood and Heartwood without Treatment (O-control) and After Bio-treatments with the Bacteria *B. subtilis*

Bacterial Treatment of Spruce		MOR	IB
Sapwood	(weeks)	(MPa)	(J/cm^2)
O-untreated***	0	61.2* (8.1)**	3.45* (0.55)**
B I	1	65.7 (5.5)	2.97 (1.49)
	3	63.0 (6.0)	3.09 (0.52)
	6	58.6 (9.7)	2.32 (0.48)
	9	65.1 (4.6)	3.88 (0.87)
B II	1	65.7 (5.5)	2.97 (1.49)
	3	66.6 (6.4)	3.86 (0.65)
	6	59.0 (5.7)	2.64 (0.39)
	9	63.1 (6.4)	3.46 (0.99)
B III	1	73.0 (5.5)	4.68 (0.98)
	3	65.7 (5.4)	4.03 (0.53)
	6	66.5 (1.8)	3.77 (0.70)
	9	63.3 (6.6)	3.35 (0.56)
B IV	1	73.0 (5.5)	4.68 (0.98)
	3	56.7 (8.9)	3.64 (1.02)
	6	67.6 (4.7)	4.19 (0.98)
	9	58.8 (3.7)	3.36 (0.73)
Heartwood	(weeks)		
O-untreated***	0	49.2 (8.7)	2.61 (0.60)
B I	9	52.7 (5.5)	2.44 (0.15)
B II	9	59.9 (4.6)	2.81 (0.65)
B III	9	47.7 (4.2)	2.48 (0.49)
B IV	9	47.9 (4.8)	2.52 (0.59)

* Arithmetic means in each series are from ten samples (n = 10).
** Numbers in the parentheses are the standard deviations.
*** O – untreated: In the case of sapwood used as reference for each week of bio-treatment; in the case of heartwood used as reference for 9 weeks of bio-treatment.

Table 3: Duncan's Tests of Significance for Permeability, Water Uptake, Impregnability Characteristics (*K*, *S*, I_S) and Mechanical Characteristics (*MOR*, *IB*) of the Bio-treated Spruce Sapwood and Heartwood

Bacterial treatment of spruce	PROPERTIES - Significance of their change after bio-treatments										
	K	S_{15min}	S_{60min}	$S_{24\text{-}hours}$	$S_{21\text{-}days}$	$I_{S\text{-}5min}$	$I_{S\text{-}15min}$	$I_{S\text{-}150min}$	*MOR*	*IB*
Sapwood										
B I 1	0.000^a	0.000^a	0.000^a	0.000^a	0.049^c	0.000^a	0.000^a	0.000^a	0.197^d	0.246^d
B I 3	0.000^a	0.000^a	0.000^a	0.000^a	0.000^a	0.000^a	0.000^a	0.029^c	0.543^d	0.372^d
B I 6	0.000^a	0.000^a	0.000^a	0.000^a	0.000^a	0.000^a	0.000^a	0.008^b	0.450^d	0.005^b
B I 9	0.000^a	0.000^a	0.000^a	0.000^a	0.000^a	0.000^a	0.000^a	0.008^b	0.255^d	0.333^d
B II 1	0.000^a	0.000^a	0.000^a	0.000^a	0.049^c	0.000^a	0.000^a	0.000^a	0.197^d	0.246^d
B II 3	0.000^a	0.000^a	0.000^a	0.000^a	0.002^b	0.000^a	0.000^a	0.011^c	0.132^d	0.343^d
B II 6	0.000^a	0.000^a	0.000^a	0.000^a	0.000^a	0.000^a	0.000^a	0.005^b	0.456^d	0.051^d
B II 9	0.000^a	0.000^a	0.000^a	0.000^a	0.471^d	0.000^a	0.000^a	0.006^b	0.538^d	0.997^d
B III 1	0.000^a	0.000^a	0.000^a	0.000^a	0.000^a	0.000^a	0.000^a	0.000^a	0.000^a	0.003^b
B III 3	0.000^a	0.000^a	0.000^a	0.000^a	0.083^d	0.000^a	0.000^a	0.000^a	0.193^d	0.193^d
B III 6	0.000^a	0.000^a	0.000^a	0.000^a	0.000^a	0.000^a	0.000^a	0.002^b	0.132^d	0.457^d
B III 9	0.000^a	0.000^a	0.000^a	0.000^a	0.023^c	0.000^a	0.000^a	0.022^c	0.519^d	0.797^d
B IV 1	0.000^a	0.000^a	0.000^a	0.000^a	0.000^a	0.000^a	0.000^a	0.000^a	0.000^a	0.003^b
B IV 3	0.000^a	0.000^a	0.000^a	0.000^a	0.000^a	0.000^a	0.000^a	0.002^b	0.164^d	0.634^d
B IV 6	0.000^a	0.000^a	0.000^a	0.000^a	0.002^b	0.000^a	0.000^a	0.000^a	0.072^d	0.093^d
B IV 9	0.000^a	0.000^a	0.000^a	0.000^a	0.000^a	0.000^a	0.000^a	0.028^c	0.452^d	0.789^d
Heartwood										
B I 9	-	0.031^c	0.027^c	0.000^a	0.058^d	0.187^d	0.148^d	0.128^d	0.286^d	0.585^d
B II 9	-	0.887^d	0.017^c	0.000^a	0.000^a	0.075^d	0.046^c	0.022^c	0.003^b	0.484^d
B III 9	-	0.034^c	0.046^c	0.000^a	0.088^d	0.131^d	0.136^d	0.163^d	0.667^d	0.658^d
B IV 9	-	0.000^a	0.000^a	0.000^a	0.000^a	0.844^d	0.845^d	0.546^d	0.692^d	0.754^d

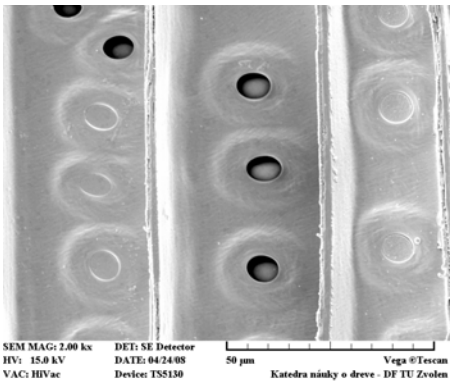
* Results are evaluated in relation to the untreated samples (see Figs. 2-4, Tabs. 1-2) at the 99.9% significance level (a), the 99% significance level (b), the 95% significance level (c), or without an evident significant difference at $p \geq 0.05$ (d)

Microscopic Analyses

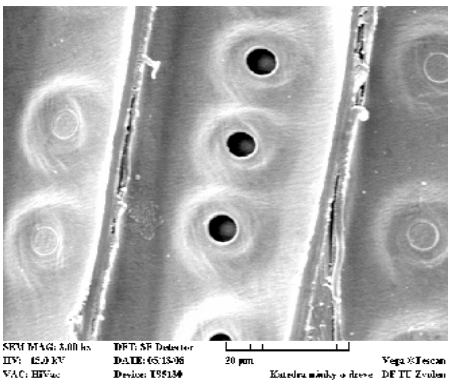
Results from the permeability, water uptake, impregnability, and strength tests were compared with results from the microscope analyses. Pits of tracheids in the bio-treated spruce sapwood were usually intensively damaged, while pits in the bio-treated spruce heartwood remained undamaged (Photos 1-6). However, cell walls of tracheids were not damaged in either sapwood or heartwood.

Proposal for Other Tests with Biological Pre-Treatments of Refractory Woods

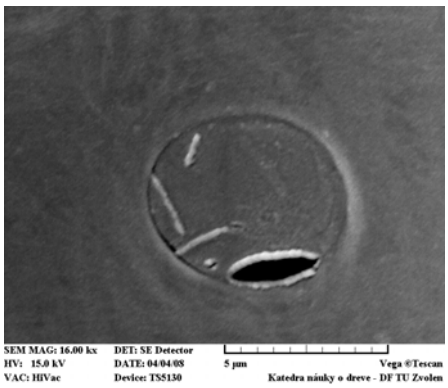
Biological pre-treatment of spruce wood, and also of other refractory species, should be sufficient for increasing the impregnability of industrial round-wooden products for exteriors, e.g. poles, palisades, or sticks. Penetration of preservatives or modification chemicals (phenol formaldehyde resins, furfuryl alcohols, acetyl anhydride, etc.) into their bio-treated sapwood zones can be quicker and deeper, and the time of impregnation can be expressively shortened.



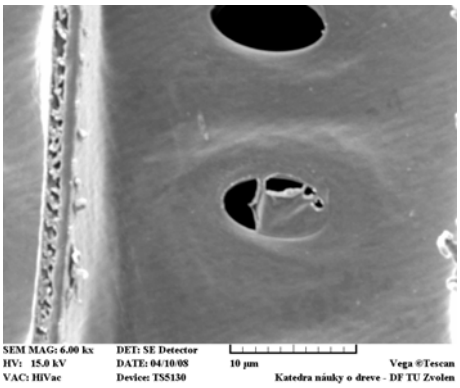
1



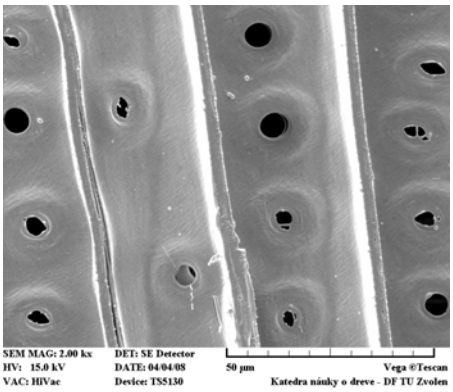
2



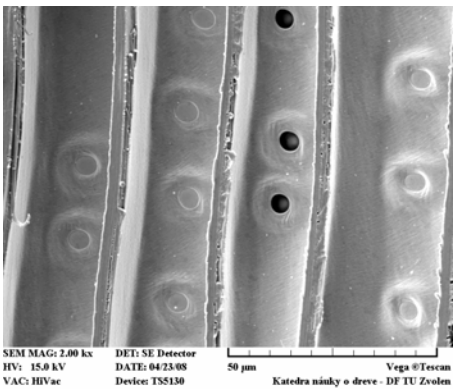
3



4



5



6

Photo 1: Non-destroyed toruses in pits of spruce sapwood (untreated spruce sapwood)
Photo 2: Non-destroyed toruses in pits of spruce heartwood (untreated spruce heartwood)
Photo 3: Detail of the bacterium *Bacillus subtilis* in pit of spruce sapwood – enzymatic degradation of the torus after 3 weeks of bio-treatment in a laboratory (type B II 3)
Photo 4: Degradation of the toruses in pits of spruce sapwood with the bacterium *B. subtilis* after 3 weeks in exterior conditions (type B IV 3)
Photo 5: The toruses in pits of spruce sapwood are fully destroyed with the bacterium *B. subtilis* after 6 weeks in a laboratory (type B II 6)
Photo 6: The toruses of spruce heartwood pits were not destroyed with *B. subtilis* even after 9 weeks in exterior conditions (type B IV 9)

With the saving of time and energy during impregnation it is also possible to obtain a higher quality (more homogenous) penetration of preservatives into wood, which results in prolongation of the service life of wooden products.

Bio-treatment of freshly cut logs with an opened system of pits in the sapwood zone has more advantages for better and faster absorption of bacteria into the entire volume of sapwood.

A disadvantage of the bacterial pre-treatment technology is probably a longer period of stagnation of raw material, because the minimal time of a quality bio-treatment should be 3 weeks. However, this time is connected with wet protection of logs in basins and their parallel biological pre-treatment. Natural lakes with higher concentration of bacteria are also useful for bio-treatment of logs (Despot 1993; Dunleavy and McQuire 1970; Unligil 1972). Water in natural lakes can contain bacteria species that degrade not only pits but also cell walls of tracheids, so the mechanical properties of wood can more extensively decrease after long-time of ponding. Bacterial treatments in basins can be better controlled and intensified by adding of useful specific species of bacteria.

Another disadvantage of such bio-treatments is also a limited time for use of this technology during the year in some territories, because the optimum temperature for the activity of more species of bacteria is about 20 to 30 °C, and at lower temperatures of water (below 15 to 20 °C) their activity decreases considerably.

Currently, the greatest problem of bacterial treatments of refractory conifers is a minimal increase of impregnability of their heartwood zones. This is caused mainly by a lignification of toruses of pits in the heartwood zone of trees already during their growth (Côte 1963; Liese and Greaves 1975). These zones fulfill mainly mechanical functions of growing trees, and so the cell walls of tracheids can be already more compact (after closing of the pits) and stronger (after lignification of the toruses). Degradation of toruses of these heartwood zones by enzymatic attacks (bacterial, fungal, etc.) must inevitably also cause degradation of cell walls in connection with a high decrease of their mechanical properties. One possibility is the use of specific cultivated species of white-rot fungi with highly controlled processes of bio-treatment (Lehringer et al. 2009; Schwarze et al. 2006; Schwarze 2008). However, this research has only begun, and applications for practical purposes are currently limited. Another possibility is the incising of wooden cell walls with traditional white-rot (*Trametes versicolor*, *Irpex lacteus*, etc.) or staining (*Ophiostoma piceae*, etc.) fungi (Yang 2009), followed by strengthening and hardening of the damaged wood with a convenient modification chemical, e.g. with a low molecular weight phenol formaldehyde resin (Wan et al. 2006).

In the future, there may be interest in the genetic modification of selected bacteria species with the aim to achieve highly controlled bio-treatment of heartwood of spruce, fir, Douglas fir, pine, and other refractory coniferous trees. However, such an approach would require the cooperation of researchers from various fields of science, and utilization of highly advanced technologies that focus on meeting the demands of the wood products industry.

The use of biological pre-treatments for logs of refractory species, from which timber used for outdoor and indoor wooden structures will be prepared, is possible only after a successful managing of increasing the impregnability of their heartwood. Sapwood zones are nearly always taken off at the preparing of angular beams, and their heartwood

zones are impregnated only on the surfaces. This situation provides favorable conditions for their attack by bio-agencies after the creation of ruptures and cracks on their surfaces. Preventive chemical protection or modification of these beams in outdoor structures cannot be perfect. Currently used mechanical pre-treatment technologies, e.g. cutting and incising, expressively decrease the aesthetical and mechanical properties of wood products. The destruction only of toruses in heartwood of conifers by specific enzymes could conserve aesthetical function of these products without decreasing of their mechanical properties.

CONCLUSIONS

Using a clear selected culture of the bacterium *Bacillus subtilis*, and use of its higher concentration during laboratory or exterior ponding of Norway spruce bolts (short logs) brought about the intensification of enzymatic degradation of pits in the sapwood tracheids. Significant increasing of the permeability characteristic (S, K, R, I_S) of the sapwood has been achieved after 1 week of spruce logs attack by the *B. subtilis*. The water uptake, the coefficient of axial permeability, and the degrees of saturation (or retention) at pressure impregnation of the sapwood zone usually increased with prolongation of bio-treatments of spruce logs – after 3, 6, and 9 weeks of action of the *B. subtilis*. On the other hand, the heartwood zone of spruce remained unchanged and pits of tracheids in this zone were not destroyed even after 9 weeks of ponding in bacterial suspensions.

On the basis of the achieved results it is evident that the action of the *B. subtillis* can be useful only for pre-treatment of poles and similar round wooden products with a possibility to achieve quicker and more homogenous preservation of their sapwood.

ACKNOWLEDGMENTS

The authors would like to thank the Grant Agency of the Slovak Republic (Project VEGA No. 1/0421/10/) for financial support in this work. They would also like to thank Dr. M. Masarykova for the microscopic analyses.

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Article submitted: March 17, 2011; Peer review completed: May 8, 2011; Revised version received and accepted: June 16, 2011; Published: June 18, 2011.