Nitrogen (N) losses from utilised agricultural areas are partly responsible for eutrophication and water quality degradation. Regardless of any measures in place for source control or reduction, a portion of nitrogen input can leak from catchments and therefore the interception and treatment of leached nitrates is needed (Passeport et al. 2013). Denitrifying bioreactors are a relatively simple, passive treatment technology for the removal of nitrate from agricultural outflows (Weigelhofer and Hein 2015). Original studies concerning this concept were carried out in Canada (Robertson and Cherry 1995) and New Zealand (Schipper and Vojvodić-Vuković 1998). The recent inclusion of woodchip bioreactors in the official nutrient reduction strategies of several US Midwestern states (Illinois, Iowa and Minnesota nutrient reduction strategies), as well as the release of the Federal USDA Natural Resources Conservation Service conservation practice standards (USDA NRCS Conservation Practice Standard No. 605, 2015), implies the growing acceptance of bioreactors as an effective tool for the treatment of nitrates in agricultural drainage (i.e. tile drainage) (Christianson and Shipper 2016).

Denitrifying bioreactors have many advantages. They are cost-effective, durable and easy to maintain, and their designs can be tailored to fit site hydrological criteria (Schipper et al. 2010a).

There are two types of this innovative technology – denitrifying beds and denitrifying walls. Denitrifying beds are often containerized treatment
systems that treat concentrated discharges from natural or tile drainage systems. Denitrifying walls are permeable reactive barriers inserted vertically into the ground to intercept groundwater flow (Schmidt and Clark 2012). Both types of denitrifying bioreactors are filled with various types of carbonaceous solids, among which wood-particle media are the most widely used material. Organic carbon (C) plays three key roles in promoting heterotrophic denitrification, which converts nitrate (NO₃⁻) to N₂ (N₂O). First, to provide an anoxic environment, second, to act as an electron donor for denitrification, and third, to promote the growth of denitrifying microorganisms (Schipper et al. 2010a).

The organic fill medium is one of the most important factors controlling the denitrification process. It must provide enough bioavailable organic carbon and maintain high hydraulic conductivity for the duration of use. Due to the need to avoid nitrogen leaching, materials with a high C:N ratio are preferred. Wood-based materials, such as sawdust and woodchips, are used most frequently because they provide constant nitrate removal rates with minimum maintenance over the long term (decades) (Robertson 2010). They also exhibit high hydraulic conductivity (van Driel et al. 2006) and a high C:N ratio – approx. 300:1 (Robertson and Anderson 1999). There is no significant difference in the denitrifying rates achieved by hard- and softwood. Hardwood may maintain its physical properties for longer due to its greater density, but is more expensive (Schipper et al. 2010b).

Other substantive factors controlling the denitrification process in a bioreactor were summarized and analysed by Addy et al. (2016). They compiled data from 26 published studies which dealt with 57 separate bioreactor units and applied meta-analysis approaches to investigate the nitrate removal rates of these units across a range of environmental and design conditions. Beds with high influent N concentrations (> 30 mg N/L) had higher nitrate removal rates than beds with intermediate (10–30 mg N/L) or low (< 0.05 mg N/L) concentrations. Addy et al. (2016) further concluded that cumulative nitrate removal in beds with hydraulic retention times (HRTs) < 6 h was significantly lower than in beds with HRTs from 6 h to 20 h and > 20 h. This problem can be solved by hydraulic control components that can adjust the extent of bypass flow during high flow events. It is well known that the rates of biochemical reactions increase with increasing temperatures (T). Beds with temperatures of less than 6°C exhibited lower nitrate removal than those at intermediate temperatures of 6°C to 16.9°C (the approximate range of Midwestern US groundwater temperatures) and temperatures which were higher than 16.9°C. Beds less than 13 months old had significantly higher nitrate removal rates than those that were 13 to 24 months old and over 25 months old. The nitrate removal rates in beds aged 13–24 months and in beds older than 25 months were not significantly different. These results concur with the suggestion of Schipper et al. (2010a) and Robertson (2010) that the rates recorded after the first year of bioreactor operation can be considered long-term rates (Addy et al. 2016).

In the initial period after denitrification bioreactors are installed, leaching of the fill media was shown to tend to release large concentrations of dissolved C, N or phosphorus (P) (Healy et al. 2012). For this reason, an approach allowing the holistic evaluation of the impact of denitrifying bioreactors on the environment is needed. Fenton et al. (2014) published a paper dealing with this issue. They defined a sustainability index (SI) which takes into account both the removal of nitrates and the release of selected pollutants. The SI is derived from the mass balances of the evaluated substances and their weightings.

The goal of our research was the laboratory testing of the suitability of various wood materials as denitrification bioreactor fill media, along with the effects of HRT, T, and inlet concentrations of nitrates on the overall impact of the reactor on the aqueous environment.

**MATERIAL AND METHODS**

**Laboratory bioreactors.** The experiment was conducted in a temperature-controlled laboratory. A stable temperature was maintained by air conditioning with a temperature setting of 0.5°C. The organic materials were placed into 0.3 m³ bioreactors (Figure 1).

The bioreactors were loaded with tap water enriched with nitrates (KNO₃), which was prepared in a dosing barrel. The NO₃⁻-N inlet concentration ranged from 8.3 to 43.1 mg/L, the water temperature from 8.3°C to 20.3°C. The water was dosed
The current flow rate was measured using a flowmeter (B METERS, Gonars, Italy). The HRTs ranged from 0.48 to 23.2 days.

The applied values of the process parameters lay in intervals that can be expected at agricultural outflows. A large set of data was attained. Each subchapter of the Results and discussion section presents the results of stages related with its topic.

A water-saturated environment was maintained in the bioreactors by a flexible pipe. Fill media included woodchip (beech, poplar, oak, spruce and larch), bark (mulch and pine + larch) and sawdust (spruce + pine) materials. The details are described in Table 1. Particle sizes were in the range from 2 mm to 20 mm. However, no significant difference in the NO$_3^-$ removal rates was found in wood particle media of different particle sizes (Schipper et al. 2010a, Schmidt and Clark 2013).

**Sampling and analyses.** Sampling was carried out on a weekly basis. Temperature and pH were measured via a Hach HQ40d multiparameter meter (Loveland, Colorado, USA). NO$_3^-$N was measured by the UV absorption method with a Hach optical Nitratax plus sc Sensor (Loveland, Colorado, USA). The chemical oxygen demand (COD) and NH$_4^+$-N were analysed by the following methods: COD – semi-micro method with potassium dichromate in acidic medium and photometric evaluation (445 nm); NH$_4^+$-N – photometric determination (425 nm) with Nessler agent.

**Data evaluation.** The sustainability index (mg/L/day) of each bioreactor was calculated according to Fenton et al. (2014) as follows:

\[
SI = a \times B(1) + b \times B(2) + c \times B(3) + d \times B(4) + \ldots
\]

Where: \( a-d \) etc., \( B(1)-B(4) \) etc. – weighting factors and the mass balances of the evaluated substances.

The mass balances \( B \) were obtained from the following equation:

\[
B = F_{\text{OUT}} - F_{\text{IN}}
\]

Where: \( F_{\text{OUT}}, F_{\text{IN}} \) – mass fluxes (F) of the evaluated substances at the outlet (OUT) and the inlet (IN), respectively, related to a volume unit of the bioreactor (mg/L/day).

From the equation, it is obvious that negative and positive balances indicate the remediation and production of the compound, respectively.

Substances and their weightings were applied based on the Czech legislation, which sets permissible annual average contamination values of surface waters (Anonymous 2015) and are shown in Table 2. The weighting factors were set in accordance with the principle that the stricter the legislative requirement, the higher the weighting factor (Fenton et al. 2014).

<table>
<thead>
<tr>
<th>Table 1. Description of fill media</th>
</tr>
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<tbody>
<tr>
<td>Parameter</td>
</tr>
<tr>
<td></td>
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<tr>
<td>-------------------------------</td>
</tr>
<tr>
<td>Bulk density (kg/m$^3$)</td>
</tr>
<tr>
<td>Dry weight (%)</td>
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<tr>
<td>Porosity (%)</td>
</tr>
</tbody>
</table>

**Table 2. The contaminants and their weighting factors used in the calculation of sustainability index**

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Permissible contamination values (Anonymous 2015) (mg/L)</th>
<th>Calculation of weightings</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO$_3^-$N</td>
<td>5.4</td>
<td>5.4/5.4 = 1</td>
</tr>
<tr>
<td>NH$_4^+$-N</td>
<td>0.23</td>
<td>5.4/0.23 = 23.5</td>
</tr>
<tr>
<td>Chemical oxygen demand</td>
<td>26</td>
<td>5.4/26 = 0.21</td>
</tr>
</tbody>
</table>
Example of SI calculation (best results – beech):

\[
\text{NO}_3^- - N: a = 1, B = -13.48 \text{ mg/L/day}; \\
\text{NH}_4^+ - N: b = 23.5, B = 0.019 \text{ mg/L/day}; \\
\text{COD: } c = 0.21, B = 11.90 \text{ mg/L/day}.
\]

\[
\text{SI} = 1 \times (-13.48) + 23.5 \times 0.019 + 0.21 \times 11.90 = -10.53 \text{ mg/L/day}
\]

Efficiency of nitrates removal (eff.) was calculated from inlet and outlet concentrations of nitrates. Bars in Figures 2, 4, 5 and 6 related to NO\textsubscript{3}^- - N, NH\textsubscript{4}^+ - N and COD, respectively, represent products of weighting factors and balances (Eq. 1).

RESULTS AND DISCUSSION

**Bioreactor fill media.** Figure 2 and Table 3 present the results of stages, where the best SIs were achieved with each of the fill media. The results were attained with the following process parameters (minimum – average – maximum): temperature 16.7 – 17.5 – 18.1°C, HRT 0.7 – 4.3 – 19.5 days, inlet NO\textsubscript{3}^- - N concentration 35.0 – 39.2 – 43.1 mg/L, respectively. These values correspond well with conditions fostering denitrification (Addy et al. 2016). The outlet pH ranged from 6.7 to 7.4, favourable for denitrification (Paul and Clark 1996). A negative SI, i.e. a positive effect on the aqueous environment, was attained in all cases. The eff. ranged from 46% to 94%. The SI was mostly affected by NO\textsubscript{3}^- - N removal and the production of organics expressed as COD, whereas the effect of NH\textsubscript{4}^+ - N was negligible. The best SI results were
shown by beech, mulch and poplar. Beech and mulch supported the denitrification to the greatest extent with nitrate removal rates higher than 13 mg/L/day, but they differed in outlet NO$_3^-$-N concentration (13.4 mg/L and 2.2 mg/L, respectively) and in eff. (65% and 94%, respectively). It can be due to the difference in COD concentrations. The beech with outlet COD concentration 27 mg/L fulfilled Czech legislative limit (Table 2), while mulch released 29 mg/L/day COD (outlet concentration 92 mg/L), which was the highest value of all tested materials. The nitrate removal rates achieved by beech and mulch were higher than the mean rates concluded for our range of the process parameters by Addy et al. (2016), but in range 2–22 mg/L/day reported by Schipper et al. (2010a). In case of poplar, worse B (NO$_3^-$-N) –7.3 mg/L/day (outlet concentration 22.0 mg/L, eff. 47%) – and low COD leaching – only 2 mg/L/day (outlet concentration 7 mg/L, in accordance with the Czech legislation) – resulted in SI comparable with mulch. Pine + larch, spruce + pine, oak and spruce showed a gradually worsening impact on the environment with decreasing nitrate removal and growing COD leaching. Negligible environmental effect was exhibited by larch. At extremely long HRT of 19.45 days, COD release was only 2.5 mg/L/day and B

Table 3. Mean outlet parameters and efficiency of nitrates removal related to the results in Figure 2

<table>
<thead>
<tr>
<th>Outlet parameter</th>
<th>Fill media</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>beech</td>
</tr>
<tr>
<td>NO$_3^-$-N (mg/L)</td>
<td>13.4</td>
</tr>
<tr>
<td>NH$_4^+$-N (mg/L)</td>
<td>0.04</td>
</tr>
<tr>
<td>Chemical oxygen demand (mg/L)</td>
<td>27</td>
</tr>
<tr>
<td>pH</td>
<td>7.33</td>
</tr>
<tr>
<td>Efficiency of NO$_3^-$-N removal (%)</td>
<td>65</td>
</tr>
</tbody>
</table>
(NO\textsubscript{3}\textsuperscript{-}-N) –1.43 mg/L/day. Spruce + pine lost its hydraulic conductivity due to compaction.

Figure 3 compares operating parameters of all stages of our experiment, i.e. stages with SI < 0 (29 stages) and SI > 0 (16 stages). Better bioreactor sustainability was achieved at higher inlet NO\textsubscript{3}\textsuperscript{-}-N, when the median NO\textsubscript{3}\textsuperscript{-}-N of stages with SI < 0 was 35 mg/L, while that of stages with SI > 0 was only 8.9 mg/L. In the tested HRT range, lower HRTs had a better effect on sustainability, with the median HRT of stages with SI < 0 being 2.1 days, whereas that of stages with SI > 0 was 6.7 days. No significant difference in the temperature of stages with different SIs was found. The effects of these parameters are analysed in detail below.

**Effect of hydraulic retention time.** Figure 4 presents average differences in B × weighting and SIs of bioreactors with various fillings measured in two subsequent stages, differing only in HRT. The results show a negligible effect on the release of NH\textsubscript{4}\textsuperscript{+}-N. At high nitrate concentrations, the decrease in HRT from 4.3 days to 1.9 days resulted in an improvement in the SI caused by the increase in the nitrate removal rate, whereas B (COD) remained almost unchanged. In contrast, the increase in HRT from 2.3 to 16.1 days resulted in an increase in the SI due to a large decrease in nitrate removal, which affected the SI much more than the drop in released COD. At low nitrate concentrations, although the change in HRT from 12.8 days to 1.4 days brought about an increase in the nitrate removal rate, the big increase in the released COD due to the high flow and low consumption of organic compounds by denitrifying bacteria led to a worsening in the SI.

As mentioned above, the best results were achieved with a relatively short median HRT of 2.1 days. However, when choosing HRT, it is necessary to take into account the inlet concentration of nitrates. Short HRTs support both nitrate removal and COD leaching. At high inlet concentrations of nitrates, the favourable impact of the high nitrate removal rate on the SI prevails over the deleterious impact of the released COD. At low concentrations, however, the deteriorating influence of the released COD on the SI may prevail over the enhancing effect of the increased nitrate removal rate.

**The effect of temperature.** The two data sets in Figure 5 present average differences in (B × weighting) and SIs of bioreactors with various fillings measured in two subsequent stages, differing in temperature. At average HRT of 1.7 days, the temperature drop brought about an average decrease of nitrate removal rate by 3.0 mg/L/day, which resulted in the deterioration of the average SI from –2.7 to –1.2 mg/L/day. At average HRT of 16.8 days, the temperature drop resulted in the improvement of the average SI from 0.2 to –0.3 mg/L/day due to the decrease in COD leaching. These results suggest that the effect of temperature on the SI depends on the HRT and probably also on the inlet concentration of nitrates. The effect of the near-zero temperatures that occur in the Czech Republic in winter have yet to be studied.

**Effect of inlet nitrate concentrations.** There are average differences in B × weighting and SIs measured in two subsequent stages at similar average HRTs and temperatures, but different inlet NO\textsubscript{3}\textsuperscript{-}-N concentrations (Figure 6). They clearly show that higher inlet concentrations of nitrates resulted in better SIs and vice versa. A change in the inlet NO\textsubscript{3}\textsuperscript{-}-N concentration from 8.9 to 43.1 mg/L caused a change in the SI from 9.0 to –5.3 mg/L/day. It should be noticed that the favourable impact of a high inlet concentration of nitrates will only occur until the denitrification capacity of the bioreactor is reached. In our experiments, the best B (NO\textsubscript{3}\textsuperscript{-}-N) was –13.5 mg/L/day. It was achieved with beech filling at inlet NO\textsubscript{3}\textsuperscript{-}-N = 43.1 mg/L, HRT = 1.58 days and T = 18°C. The resulting SI was –10.5 mg/L/day due to the decrease in COD leaching.

The effects of HRTs, temperatures and inlet concentrations of nitrates are mutually interdependent. Further research should lead to multidimensional graphs expressing these relationships.

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