SUITABILITY OF DENITRIFYING WOODCHIP BIOREACTOR OUTFLOWS FOR USE IN IRRIGATION

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Abstract: Denitrifying bioreactors are an innovative technology aimed at lowering high nitrate concentrations in agricultural runoff *in situ*. The most important component is a biodegradable filtration medium that serves as an organic carbon source for denitrifying bacteria, which reduces nitrates to nitrogen gases. However, undesirable excessive leaching of organic compounds from such bioreactor fillings can occur (especially in the start-up phase) with an adverse effect on the sensitive aquatic environment. The aim of this paper is to assess the possibility of using organics-rich water for irrigation. Static leaching tests and dynamic column tests were performed with various denitrifying bioreactor fill media to evaluate the leachability of organic substances (via the determination of chemical oxygen demand – COD, biochemical oxygen demand – BOD, and total organic carbon – TOC). The toxicities of the leachates were assessed via two terrestrial plant bioassays (*Sinapis alba* and *Raphanus sativus*). The tests with *Sinapis alba* indicate that some wood species (oak and acacia) exhibit higher toxicity, and that pretreatment by drying has a negative effect. A correlation was found between toxicity to *Sinapis alba* and COD, BOD, and TOC. As regards the dynamic tests, the concentration of organic compounds decreased with operation time, while toxicity to *Sinapis alba* increased. No toxic effect on *Raphanus sativus* was observed, and the toxic effect on *Sinapis alba* was slight. The results suggest that drained area irrigation using outflows from denitrifying bioreactors could be possible; however, such a decision would require more complex research involving more plant species.

Key Words: denitrifying bioreactor, wood chips, irrigation, ecotoxicity, terrestrial plants

INTRODUCTION

The contamination of the aquatic environment with nitrates has become a global problem in the last few decades due to its ability to cause eutrophication, toxic algal blooms, hypoxia and habitat deterioration (Galloway et al. 2003). It often has anthropogenic sources, with agriculture making a particularly significant contribution to the problem. The prevention of the pollution of water bodies in this way is covered by the European Directive concerning the protection of waters against nitrate pollution from agricultural sources (Council of the European Communities 1991).

Denitrifying bioreactors are a relatively simple treatment technology for the removal of nitrates from agricultural outflows. The first studies concerning this concept came from Canada and New Zealand, later followed by work from the USA (Christianson and Schipper 2016). Denitrifying bioreactors operate by causing nitrate-rich water to flow through an organic material rich in bioavailable carbon. This promotes heterotrophic denitrification, a process which converts nitrates (NO_3^-) into N-gases (Schipper et al. 2010).

Although denitrifying bioreactors have been used for a relatively long time, there are still a few unsolved problems, especially the excessive leaching of organic compounds during the start-up phase (Cameron and Schipper 2010). The release of organic substances can cause dissolved oxygen depletion in receiving waters and adversely affect biota (Schipper et al. 2010). Svensson et al. (2014a) examined dissolved organic carbon (DOC), biochemical oxygen demand (BOD), pH, colour, phenols, tannins and lignin in sawdust leachates of oak, pine, maple, and beech. Statistically significant differences among the tree species were found. These results indicate the need to consider wood species when assessing potential environmental effects.

Malá et al. (2016) focused on the excessive leaching in the start-up phase. They performed column leachability tests with different types of wood chips and shavings. The leachates from most of the materials showed high chemical oxygen demand (COD) and BOD at the beginning of the tests, with a distinct decrease after nine weeks. Some studies point to the fact that wood leachates (e.g. denitrifying bioreactor outflow) can be toxic to aquatic organisms.

Leachate composition depends on the structure as well as the physical and chemical properties of the wood species. Trees are known to contain water-soluble phenolic compounds and mostly hydrolysable tannins or ellagitannins, such as esters of gallic acid. Some of the organic compounds present in wood leachate, like tannins, lignins, phenols, tropolones, and resin acids, can contribute to leachate toxicity (Svensson et al. 2014b, Samis et al. 1999). Phenols and resin acids such as isopimaric (IA) and dehydroabietic (DHAA) acids represent the greatest risk to aquatic life. IA is the most toxic, but also the rarest of the group of acutely toxic resin acids. DHAA is one of the least toxic resin acids, but is often mentioned in pulp & paper toxicology literature because it is the most soluble resin acid and can be reduced to retene, which is toxic to aquatic organisms. (Makris and Banerjee 2002)

Rex et al. (2016) assessed six types of wood chips (aspen, lodgepole pine, hybrid white spruce, black spruce, and two mixtures). All of the studied wood chips produced leachate that was toxic to *Vibrio fischeri* in Microtox^{TM}. Aspen chips produced the most acidic leachate, with higher organic, phenolic and ammonia concentrations compared to the coniferous and mixed samples. Resin acid concentrations for IA and DHAA were lowest in aspen, however. This indicates that either the high organic component concentration or the combination of organic compounds and resin acids is responsible for the toxicity response. Libralato et al. (2007) showed that leachates from wood species were more toxic to two saltwater organisms after 24 h of leaching than after 72 h, suggesting that whatever the components responsible for toxicity were, they were short lived. Libralato et al. (2007) thought that the toxic effect is caused by naturally occurring extractives, including aldehydes, phenols, terpinene, camphene and pinene.

TOC and COD provide relevant information about the total amount of organic compounds, but they are not informative with regard to organic compound distribution (Svensson et al. 2014b). However, a correlation between organics concentration and toxicity can be found. For example, Kannepalli et al. (2016), who focused on the leachate quality of mulch from wood materials (such as whole trees, tree trunks, and tree stumps), observed a significant negative correlation between the stage delay concentrations of zebrafish (*Danio rerio*) embryos and COD. The negative slope for this relationship indicates that with increased COD concentration in a sample, the volume causing developmental delay decreased (higher toxicity). However, Libralato et al. (2007), who investigated the potential toxic effects of wood leachates (oak, Norway spruce and three tropical species) on two saltwater organisms (the brine shrimp *Artemia franciscana*, and the embryos of the oyster *Crassostrea gigas*), did not find any significant correlation ($p < 0.05$) between physical and chemical parameters (dissolved oxygen, pH, COD) and ecotoxicological data.

Even if the most suitable bioreactor fill medium is selected and operating conditions are optimized, the leaching of organic compounds can still be too high. One possible way to protect a sensitive aquatic ecosystem of recipient is to use outflow water to irrigate a drained area in the first period of bioreactor operation. However, the effect of denitrifying bioreactor outflows on terrestrial organisms has been studied even more rarely than aquatic ecotoxicity. It is well known that tannins are toxic to microorganisms and ruminant animals; however, no information has been found about the toxic effect of tannins on plants, although they are not expected to have toxic effects on seeds (Svensson et al. 2014b).

For example, Svensson *et al.* (2014b) observed that oak wood leachate had no effect on *Lactuca sativa* (germination test). Feldmane (2010) investigated the influence of woodchip mulch on the growth and first yield of sour cherries. He measured the tree height, sum of shoot length, canopy volume and yield. The results suggest that the use of woodchip mulch tended to decrease growth in the first two growing years, though it significantly advanced growth in the third year. However, the trees treated with woodchip mulch had a lower yield than those left untreated.

MATERIAL AND METHODS

Static leaching tests and dynamic column tests were performed to evaluate the leachability of organic substances from several wood materials that can be used as denitrifying bioreactor fill media. Besides the types of organic substances present, the leachates were assessed for toxicity to terrestrial plants.

Static leaching tests

Static leaching tests were conducted with chips fabricated (size fraction 1.4–22.4 mm) from six wood materials, these being a mixture of pine and larch bark, and poplar, beech, spruce, oak, and acacia wood. The materials were tested in their original state, with dry matter content ranging between 80 and 90%. The beech and spruce were also pretreated by drying (6 h, 105 °C).

The tests consisted of 24 hours of leaching in a rotating shaker (5 rpm). The leaching was carried out using deionized water under laboratory conditions with a solid to water ratio of 1/10.

Dynamic column tests

The dynamic column tests were conducted with two mulches, spruce and pine, each placed in two 0.3 m^3 laboratory columns. The bioreactors were filled with tap water enriched with nitrates (KNO_3). The water was dosed at 12 intervals per day and the hydraulic retention time was approx. 1 day. The length of the dynamic test was 4 weeks and samples for chemical analyses were taken on the 4^{th} and 28^{th} day of operation. The water temperature ranged from 13.0 to 17.0 °C, and the concentration of NO_3^- – N was 26.3 mg/l at the start (4^{th} day) and 20.5 mg/l at the end (28^{th} day).

Chemical properties

Organic substances were determined in the samples, which were not filtered, especially COD, BOD, and total organic carbon (TOC). The laboratory analyses were performed as follows: COD – semi-micro method with potassium dichromate and photometric evaluation; BOD – standard dilution method; TOC – purging method using Hach TOC cuvette tests.

Ecotoxicological bioassays

Two ecotoxicological bioassays were carried out – an acute bioassay with the seeds of *Sinapis alba* (white mustard), and a long-term bioassay with *Raphanus sativus* plants (cultivated radish). The *Sinapis alba* bioassay was performed as a 72-h test according to methodology set out by the Czech Ministry of the Environment. (MŽP 2007) The percentage of root growth inhibition was calculated. The *Raphanus sativus* bioassay was performed as a modified test according to Test No. 208 (OECD 2006). The test plants were grown in twenty 200 mL plastic pots filled with fertilized commercial potting soil under laboratory conditions – 3 plants in each pot. In the case of the static tests, fresh leachates were prepared every week, and the length of the test was 5 weeks. For the dynamic tests, the outflow from the columns was collected periodically and the length of the test was 4 weeks. The germinability and external appearance of the plants were assessed during the test, and the dry shoot weight was measured at the end. The results were compared with those for the control plants, which were grown under the same conditions but without the application of leachate.

Statistical evaluation

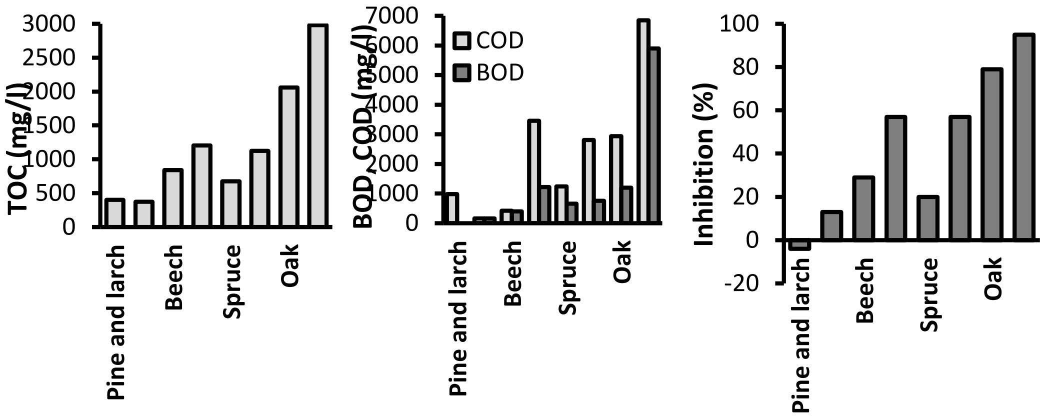
For the determination of the relationship between toxicity and concentrations of organics, *Sinapis alba* inhibition was converted to probits for linearization, and then linear regression was applied (only positive inhibition values were used). For the detection of differences among *Raphanus sativus* dry shoot weight means, data was statistically evaluated by one-way analysis of variance (ANOVA) with Dunnett's post–hoc test ($P < 0.05$).

RESULTS AND DISCUSSION

Static leaching tests

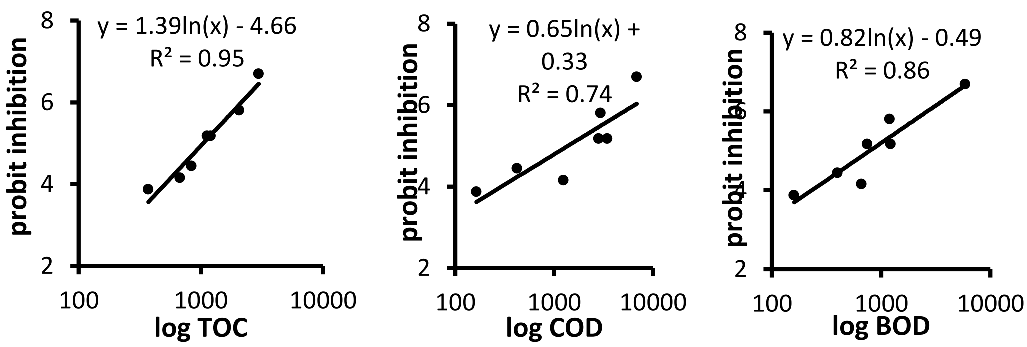
In the case of static leaching tests, TOC, BOD, COD and toxicity to *Sinapis alba* vary according to the wood species used and the application of pretreatment (Figure 1). The results confirm the statement by Svensson et al. (2014a), who determined that there was a difference between the leachates obtained from various wood species. Higher leachability of organic compounds and toxicity is shown in the case of pretreated (dried) materials in comparison with those left untreated. The levels of toxicity to *Sinapis alba* ranged from nontoxicity (lower than 30% inhibition – the pine and larch mixture, and poplar, beech, and spruce) to toxicity (higher than 50% inhibition – non-treated oak and acacia, and dried beech and spruce). However, the static leaching tests represent maximum leachability, not the real situation.

Figure 1 Results of static leaching tests: TOC, BOD, COD, and toxicity to Sinapis alba (the BOD of pine and larch was not determined)



The results suggest the existence of a relationship between toxicity to *Sinapis alba* and TOC, COD, and BOD (Figure 2). A positive linear relationship was found between toxicity and all measured parameters – TOC (correlation coefficient r = 0.97), COD (r = 0.86), and BOD (r = 0.93). Despite the fact that the studied samples were from different types of wood with varied leachability and toxicity, the data showed a strong correlation, which was found to be significant at $p < 0.05$. The results correspond with those of Kannepalli et al. (2016), who found a correlation between COD and toxicity, and also correlations between COD and BOD and potentially toxic phenolic compounds. However, there may be differences in toxic effect depending on the tested organism species, as stated by Libralato et al. (2007).

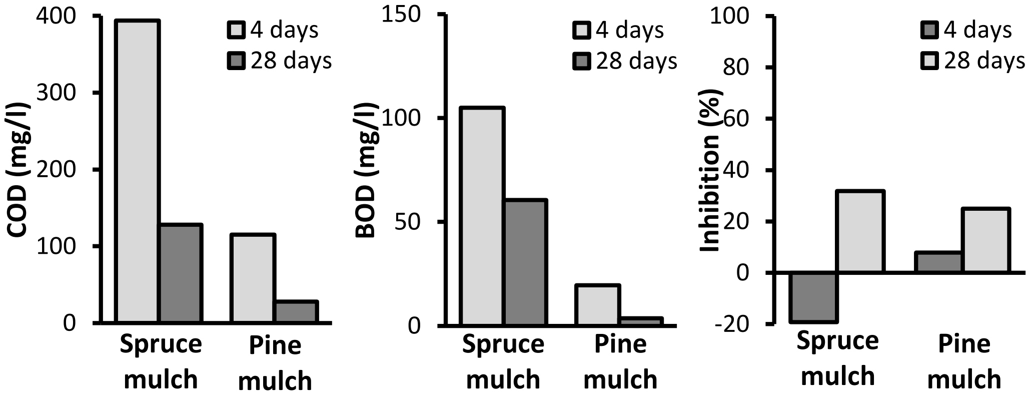
Figure 2 Relation between toxicity to Sinapis alba and TOC, COD, and BOD



Dynamic column tests

The results of the dynamic column tests, where the data from the 4th and 28th day of operation were compared, are shown in Figure 3. These tests simulate the start-up phase of a denitrifying bioreactor. While organic compounds decreased with time of operation (which corresponds with the findings of Cameron and Schipper (2010) and Malá et al. (2016)), toxicity to *Sinapis alba* slightly rose. Inhibition increased from -19 to 32% and from 8 to 25% in the case of spruce and pine mulch, respectively. However, although some changes in toxicity were observed, three of the samples were non-toxic; only spruce mulch leachate slightly exceeded the limit for non-toxic materials (30% inhibition) after 28 days. It indicates potential changes occur in the quality of leachates over time, leading to the leaching of lower concentrations of more toxic organic compounds. This topic requires more detailed research with more materials, longer time periods and more extensive sampling.

Figure 3 Results of dynamic column tests: Values for COD, BOD and toxicity to Sinapis alba (average values)



The long-term effect (4/5 weeks) of irrigation on plant growth was investigated with *Raphanus sativus* seeds, which were watered with fresh static leachates or column outflows. In both cases, no significant difference was found between these and the control seeds ($p < 0.05$), and there were no visual differences during growth. The inhibition values are shown in Table 1. These results confirmed the research of Svensson et al. (2014b), who did not find any effect of oak wood leachate on *Lactuca sativa* in a similar test.

Table 1 Toxicity of the leachates to Raphanus sativus

Static tests		Dynamic tests	
Material	Inhibition	Material	Inhibition
Pine and larch	-8.8%	Spruce mulch	4.7%
Poplar	-5.1%	Pine mulch	7.9%

CONCLUSION

Even though static leaching tests do not simulate a real situation, they are a usable method for the comparison of various materials. On the other hand, dynamic column tests provide a simulation of real denitrifying bioreactor processes, although under more complex conditions.

The static leaching tests indicated the toxicity of some wood species leachates (oak and acacia) to *Sinapis alba* and the negative effect of pretreatment of the wood by drying. The leachates of pine and larch, poplar, beech, and spruce were non-toxic. A significant relationship between toxicity to *Sinapis alba* and TOC, COD, and BOD was found. These results pointed out the importance of wood species selection and the state of the material for the use of woodchips in denitrifying bioreactors.

Despite the fact that the concentration of organic compounds released during dynamic tests decreased with operation time, toxicity to *Sinapis alba* slightly increased. This indicates that long-term leaching can cause the release of more complex and stable organics that may show higher toxicity.

The lack of any observed toxic effect on *Raphanus sativus* suggests drained areas could be irrigated using outflows from denitrifying bioreactors. However, although the *Sinapis alba* bioassays performed on the outflows indicate a slight toxic effect, the evaluation of the possibility of irrigation using such outflows requires more complex research with more plant species and stages of growth.

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