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START-UP PHASE OF DENITRIFYING BIOREACTORS USED FOR AGRICULTURAL RUNOFF TREATMENT

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Abstract

Denitrifying bioreactors are a useful passive treatment technology for the removal of nitrates from agricultural runoff. The start-up phase is the most critical period of their operation. In this phase, denitrification rates gradually increase and organic compounds, which are necessary for denitrification, are released from the bioreactor fill media in excessive amounts. The aim of our study was to evaluate the start-up phase of laboratory denitrifying bioreactors filled with six wood-based materials. The effluent quality of all bioreactors achieved a steady state after max. 9 weeks. The denitrification rates at the end of this period ranged from 0.16 to 5.8 g/m^3/d. Initial outlet chemical oxygen demand and biochemical oxygen demand leaching rates, which were in the hundreds and tenths of g/m^3/d, respectively, decreased below 55 and 30 g/m^3/d, respectively. Based on the results reported both for the leaching of organic compounds and the removal of nitrates, poplar seems to be the most suitable denitrifying bioreactor fill medium out of all the tested materials.

Key words: *agricultural runoff, denitrifying bioreactor, organic compound leaching, start-up phase, wood-based. materials.*

INTRODUCTION

Excessive application of fertilizers and animal manure makes agriculture one of the main sources of nitrogen in the aquatic environment and contributes to nitrate contamination worldwide. The high level of nitrates in surface waters may lead to eutrophication, toxic algal blooms, hypoxia, and habitat deterioration (Galloway et al., 2003).
Denitrifying bioreactors are a useful passive treatment technology for nitrate removal from agricultural outflow. They can take the form of beds (containerized systems treating concentrated discharge) or walls (permeable reactive barriers intercepting groundwater flow) (Schmidt and Clark, 2012). They are filled with carbonaceous material, usually wood-based, which releases organic C over a long period and thus fosters heterotrophic denitrification, which converts nitrates to gaseous nitrogen or nitrous oxide (Schipper et al., 2010).
Processes based on heterotrophic denitrification rank among the most promising and frequently used nitrate removal approaches (Robertson, 2010). Denitrifying bioreactors were first studied in Canada (Robertson and Cherry, 1995) and New Zealand (Schipper and Vojvodić-Vuković, 1998). Currently, the technology is in use as part of the official nutrient reduction strategies of several US Midwestern states (Illinois, Iowa and Minnesota nutrient reduction strategies) and the Federal USDA Natural Resources Conservation Service conservation practice standard (USDA NRCS Conservation Practice Standard No. 605) (Christianson and Shipper, 2016).
The main advantage of this technology is its simplicity and long-term high efficiency. Denitrifying bioreactors are capable of removing up to 100% of nitrates. They are easy to assemble and maintain, which makes them cost effective. They are durable–they can last for a minimum of 15 years with minimum maintenance and without requiring replenishment of the fill medium (Schipper et al., 2010).
The fill medium and process parameters are factors controlling the denitrification process in bioreactors. Among the various types of organic fill media, wood-particle materials are the most widely used (Schipper et al., 2010). These media are suitable because they provide consistent NO_3-N removal rates (1–20 g/m^3/d) over the long term (Robertson, 2010) and

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exhibit high hydraulic conductivity (van Driel et al., 2006) and a high C:N ratio of approx. 300:1 (Robertson and Anderson, 1999). The process parameters of denitrifying bioreactors, including inlet NO_3-N concentration, hydraulic retention time (HRT), and temperature, were summarized and analysed along with their influence on the denitrification rate by Addy et al. (2016). They applied meta-analysis approaches to data from 26 published studies which dealt with 57 separate bioreactor units. They concluded that the NO_3-N removal rate rises with increasing inlet NO_3-N concentration, and that furthermore it is fostered by a sufficient HRT (denitrifying units with a HRT higher than 6 h have greater NO_3-N removal rates than units with a lower HRT) and sufficient temperature (denitrifying units with a temperature that is greater than 6°C exhibit higher NO_3-N removal rates than units with a lower temperature).

The start-up phase of a bioreactor is the most critical because of the potential release of large concentrations of easily soluble organic carbon and NH_4-N via leaching of the fill medium (Robertson and Anderson, 1999; Gilbert et al., 2008; Cameron and Schipper, 2010; Schipper et al., 2010). The release of organic substances can cause dissolved oxygen depletion in receiving waters and adversely affect biota (Schipper et al., 2010). Certain organic compounds occurring in wood leachate (namely phenolic compounds, tannin and lignin) can, at higher levels, be toxic to aquatic organisms (Schmidt and Clark, 2013). The initial period contrasts with steady-state conditions, when leaching of the fill media is assumed to be negligible (Healy et al., 2012). Cameron and Schipper (2010) reported a drop in NH_4–N and biochemical oxygen demand (BOD) leaching concentrations from wood media to below 0.3 mg/L and 10 mg/L, respectively, within two months after commissioning. Longer HRTs and higher temperatures generally produced higher concentrations of NH_4–N and BOD, probably because the higher temperatures supported faster microbial decomposition of carbon media and low flow rates led to elevated concentrations. To minimise the release of NH_4–N and BOD into vulnerable environments during the start-up phase, temperature management measures, i.e. commissioning the denitrification bioreactor during winter, and HRT management measures, i.e. flow rate or hydraulic gradient alteration, which should be performed together with the selection of appropriate fill media, can be employed (Cameron and Schipper, 2010).

The aim of this study was the evaluation of the start-up phase of laboratory denitrifying bioreactors filled with six wood-based materials. The evaluation was based both on nitrate removal and media leaching assessment. The effect of process parameters was also considered.

MATERIALS AND METHODS

Denitrifying laboratory columns

The tests were conducted in a temperature controlled laboratory with six vertical cylindrical bioreactors, each 1,000 mm high, with a diameter of 300 mm. The bioreactors were filled with a weighed amount of organic material. During the test, the bioreactors were loaded with dechlorinated tap water enriched with nitrates (KNO_3). The water flowed vertically from top to bottom. The location of the outlet pipe provided a water saturated environment in the bioreactors.

The effluent quality of all bioreactors achieved a steady state after a maximum of 9 weeks, which agrees with the findings of (Cameron and Schipper, 2010). Therefore, the first nine weeks of operation of the bioreactors were considered to be the start-up phase. Table 1 shows the process parameters of the bioreactors at the end of the start-up phase.

Table 1. Process parameters of the bioreactors at the end of the start-up phase

	Sawdust mixture *	Bark mulch **	Woodchips			
			Larch	Oak	Poplar	Spruce
HRT, d	6.60	2.50	2.04	2.47	13.2	5.95
Temperature, °C	17	17	17	17	9	19
Inlet NO_3-N, mg/L	34.7	34.7	34.2	34.2	38.6	8.6

* Sawdust from a mixture of various trees.
** Commercially available bark mulch.

It is obvious that the operating parameters differed. This is due to the fact that the bioreactors were not started up simultaneously. Three materials were tested at low HRTs (2 to

2.5 d), one material at a long HRT (13.2 d), and in two cases the HRTs were in-between (6 and 6.6 d). Five materials were tested at an ambient temperature of 17 or 19°C; only poplar woodchips were tested at a temperature that was 9°C. Five materials were tested at an inlet water NO_3-N concentration of 34 to 39 mg/L, while the concentration for spruce woodchips was 8.6 mg/L.

Water sampling and analysis

The frequency of inlet and outlet sampling was set to one week. Temperature was measured via a portable probe and the data were recorded by a Hach HQ40d multi-parameter meter. The chemical oxygen demand (COD), BOD, NH_4-N and NO_3-N were analysed by the following methods: COD–semi-micro method with potassium dichromate and photometric evaluation; BOD–dilution and seeding method with allylthiourea addition and five-day incubation time; NH_4-N–photometric determination with Nessler agent; NO_3-N–UV absorption method with a Hach Nitratax plus sc Sensor.

Data evaluation

Nitrate removal was evaluated based both on denitrification rates and removal efficiencies. Denitrification rates (*DR*) in g/m^3/d were calculated from inlet and outlet NO_3-N concentration differences:

$$DR = \frac{\Delta c(\mathrm{NO_3{-}N}) \cdot Q}{V} \quad (1)$$

where: Δc(NO_3-N) is the difference in NO_3-N inlet and outlet concentrations (mg/L), Q is the water flow rate (L/d) and V is the bioreactor filling volume (L). Removal efficiency was calculated from inlet and outlet NO_3-N concentrations.

Release of COD, BOD, and NH_4-N was evaluated based both on concentrations and leaching rates. Leaching rates (*LR*) in g/m^3/d were calculated as mass flow rates in relation to bioreactor filling volume:

$$LR = \frac{c \cdot Q}{V} \quad (2)$$

where: c is the outlet concentration (mg/L), Q is the water flow rate (L/d) and V is the bioreactor filling volume (L).

To estimate total COD leaching, COD leaching rates were fitted to an exponential decay model originally used by Schmidt and Clark (2013) to describe the decrease over time of dissolved organic carbon export rates from denitrifying bioreactor fill media. The model's equation was as follows:

$$LR_{\mathrm{COD}}(t) = LR_{\mathrm{COD}}(0) \cdot e^{-rt} + \theta \quad (3)$$

where: $LR_{\mathrm{COD}}(t)$ and $LR_{\mathrm{COD}}(0)$ are the COD leaching rate (g/m^3/d) in time t and in the initial time, respectively, r is the exponential rate constant and θ is the asymptote rate (g/m^3/d). COD concentration and COD leaching rate were measured and calculated, respectively, for an extended period of time (18 weeks).

The variable r was determined using Microsoft Excel software. The variable θ was manually fitted as the average COD leaching rate in the final three sampling events.

RESULTS AND DISCUSSIONS

Nitrate removal

Denitrification rates gradually increased during the 9 weeks of the bioreactors' start-up phase (Figure 1).

After one week of operation, *DRs* varied from 0 g/m^3/d (spruce) to 2.2 g/m^3/d (mulch). The subsequent increase in *DRs* over time was interleaved by a sigmoid curve.

The smallest and greatest rise in *DR* during the start-up phase was shown by mulch (1.2 times) and sawdust (11.8 times), respectively. However, the highest *DR* (9.04 g/m^3/d) was achieved by oak at the eighth week of operation.

The denitrification rates and NO_3-N removal efficiencies which were achieved by the studied denitrifying bioreactor fill media at the end of the start-up phase are summarised in table 2.

The denitrification rates varied from 0.16 g/m^3/d (spruce) to 5.8 g/m^3/d (oak) with an average of 2.4 g/m^3/d.

All achieved denitrification rates correspond well with the findings of Christianson et al. (2012) and David et al. (2016) - 0.38-7.76 g/m^3/d and 1.2-11 g/m^3/d, respectively, though they are rather lower compared with the general range of 2-22 g/m^3/d published in (Shipper et al., 2010).

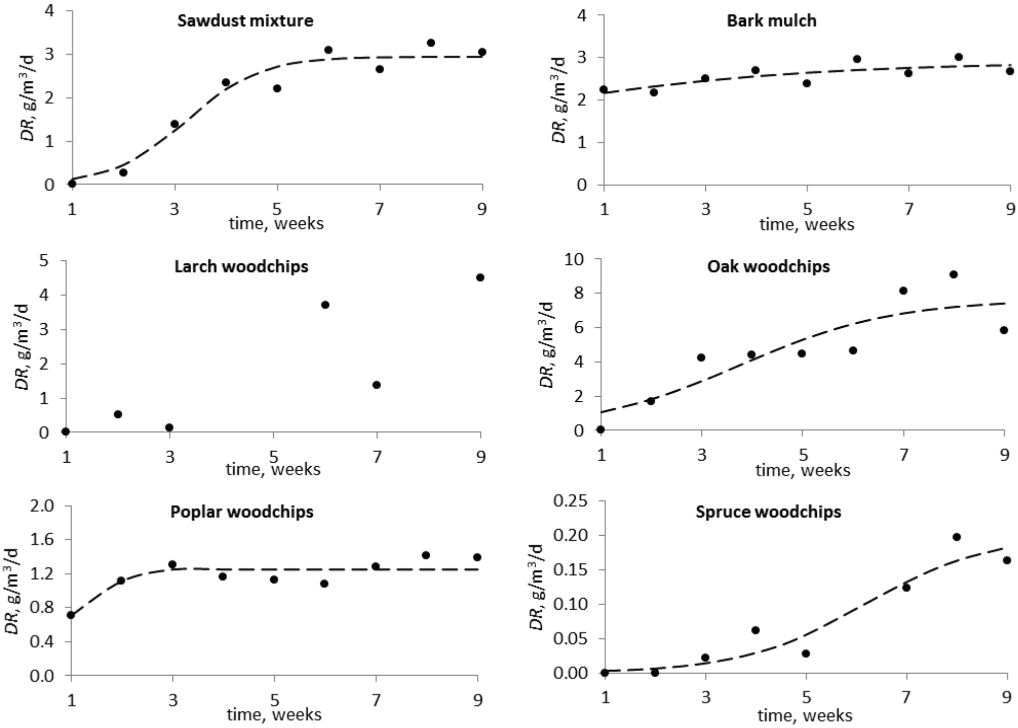


Figure 1. Changes in *DR* during the bioreactors' start-up phase

Table 2. The denitrification rates and NO_3-N removal efficiencies at the end of the start-up phase of denitrifying bioreactors filled with various wood-based media

	Sawdust	Mulch	Larch	Oak	Poplar	Spruce
Denitrification rate, $g/m^3/d$	3.0	2.7	1.4	5.8	1.4	0.16
Outlet NO_3-N, mg/L	7.4	4.7	31	13	4.6	5.7
NO_3-N removal efficiency, %	79	86	9	63	88	34

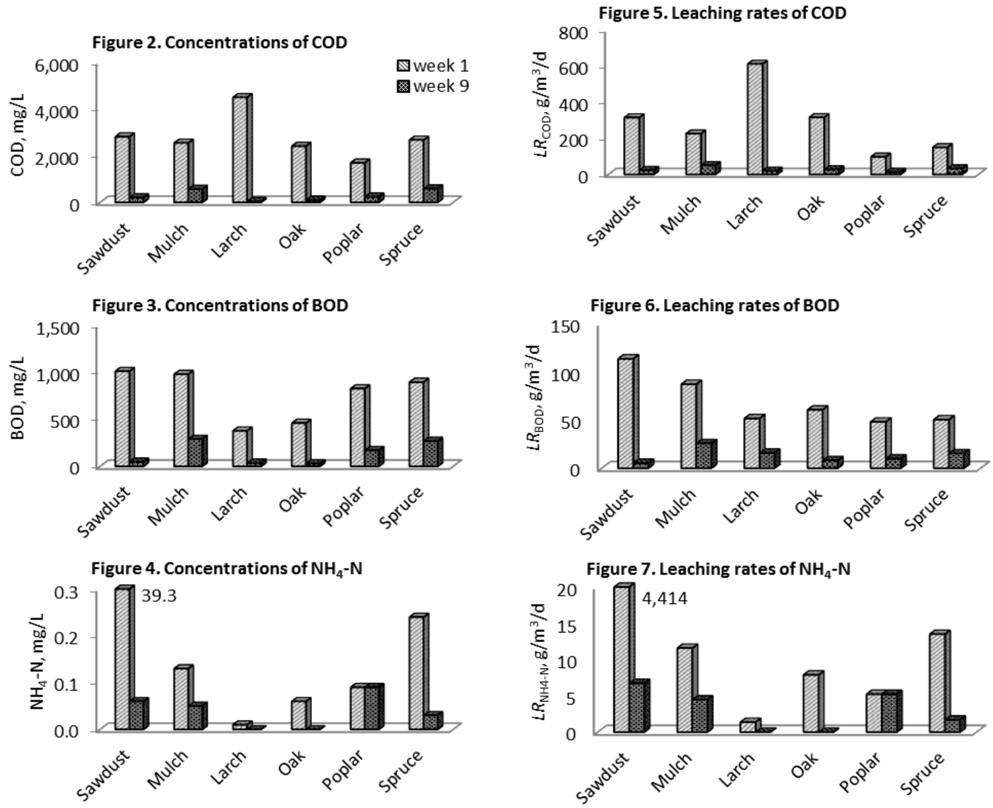
From the fact that the highest *DR* (oak) and the second lowest *DR* (larch) were achieved with the same process parameters (HRT 2.0-2.5 d, inlet NO_3-N 34.2 mg/L, and temperature 17°C) it is obvious that the *DR* at the end of the start-up phase largely depended on the fill medium. The lowest *DR* was reported for spruce, which was, unlike the other materials, used with a low inlet NO_3-N concentration (8.6 mg/L). This confirms the conclusions of Addy et al. (2016) that the *DR* increases with increasing inlet NO_3-N concentration. High nitrate removal efficiency is fostered by long HRT (Hoover et al., 2015). However, long HRT is also associated with low flow rate and thus can lead to low *DR* due to a low inlet NO_3-N mass flow rate (Formula 1). This was probably the case with poplar, which achieved the highest NO_3-N removal efficiency of all studied media (88%), but low *DR* (1.4 $g/m^3/d$). The low *DR* of poplar could also be partly caused by low temperature (9°C).The lowest and highest outlet NO_3-N concentrations were achieved with poplar (4.6 mg/L) and larch (31 mg/L), which correspond to the highest (88%) and lowest (only 9%) NO_3-N removal efficiencies respectively, achieved by these media.

It can be concluded that good results were shown by four of the six studied wood-based media, namely sawdust, mulch, oak (with the highest denitrification rate, 5.8 $g/m^3/d$), and poplar (with the highest NO_3-N removal efficiency, 88%, despite the low temperature of 9°C). In contrast, unfavourable results were achieved with larch (denitrification rate 1.4 $g/m^3/d$ and NO_3-N removal efficiency 9%) and spruce (denitrification rate 0.16 g/m^3/d and NO_3-N removal efficiency 34%). Therefore, based on the denitrification rates achieved at the end of the start-up phase it can be said that these two media do not seem to be suitable for use in denitrifying bioreactors.

Denitrifying bioreactor media leaching

The study focused on the release of organic substances (expressed as COD and BOD) and NH_4-N, because increased concentrations of these compounds in bioreactor outlet can threaten the water quality of the recipient.

Figures 2 - 7 show changes in concentrations and leaching rates of COD, BOD, and NH_4-N from the beginning to the end of the start-up phase, i.e. from the first to the ninth week of bioreactor operation.



Figures 2, 3, 4 ,5, 6, 7. Changes in COD, BOD and NH_4-N concentrations and leaching rates from the beginning to the end of the start-up phase. Light and dark bars represent values in the first and ninth week of bioreactors' operation, respectively.

It can be seen from Figures 2 to 7 that although there were differences among the concentrations and leaching rates of the released substances, both concentrations and leaching rates decreased substantially during the start-up phase.

At the beginning of the experiment, the highest bioreactor outlet COD concentration was shown by larch – 4,487 mg/L, while the COD values measured for the other materials were comparable (1,694–2,809 mg/L). Interestingly, the larch also showed the biggest decrease in COD concentration, as outlet COD was only 47 mg/L in the ninth week of the experiment. The outlet COD measured for the other materials ranged from 101 to 590 mg/L (Figure 2).

The COD leaching rates followed a similar pattern, with the highest value at the beginning of the experiment calculated for larch (613 g/m^3/d) and the lowest for poplar (99 g/m^3/d). At the end of the start-up phase, the COD leaching rates were much lower and more balanced compared with the first week and ranged from 14 g/m^3/d (poplar) to 51 g/m^3/d (mulch) (Figure 5).

In the case of outlet BOD, the differences among the materials were not so large. The initial outlet BOD concentrations ranged from 378 mg/L (larch) to 1,010 mg/L (sawdust), and the initial BOD leaching rates from 48 g/m^3/d (poplar) to 113 g/m^3/d (sawdust). During the start-up phase, the outlet BOD concentrations decreased to 30–289 mg/L (oak–mulch) and the leaching rates to 5.4–26 g/m^3/d (sawdust–mulch) (Figures 3 and 6).

The lowest initial COD and BOD leaching rates were reported for poplar (99 and 48 g/m^3/d, respectively) (Figures 5 and 6). The HRT of this bioreactor was high (13.2 d) in comparison with other experimental units, which had HRTs ranging from 2.0 to 6.6 d (Table 1). This may suggest that although high HRTs can foster an increase in concentrations of released compounds (Cameron and Schipper, 2010), low flow rates help to reduce leaching rates in the start-up phase, which is favourable for the receiving stream. The bioreactor filled with poplar woodchips that showed the best results differed from other bioreactors in its low operating temperature, which was 9°C (Table 1). It confirms the findings of Cameron and Schipper (2010) that low temperatures are favourable for the start-up phase, as they aid in slowing down the decomposition and leaching of the fill media.

There was a big difference between the NH_4-N released from sawdust and from all the other materials at the beginning of the experiment. While sawdust showed an NH_4-N outlet concentration of 39.3 mg/L and a leaching rate of 4,414 mg/m^3/d in the first week, the second highest values (spruce) were only 0.24 mg/L and 13.5 mg/m^3/d, respectively. The high NH_4-N leaching rate from the sawdust could probably be explained by that material's consistency and large specific surface, which also applied in the case of the high BOD release, although in the latter case the difference between it and the other fill media was not so great. In the ninth week, the outlet NH_4-N concentrations of all bioreactors were below 0.1 mg/L and leaching rates were below 7 mg/m^3/d (Figures 4 and 7).

To estimate the total COD leached, COD leaching rates were fitted to an exponential decay model (Figure 9). The variables of the model, $LR_{COD}(0)$, r and θ, are summarized in Table 3, which also includes the determination coefficients. These varied from 0.98 (sawdust) to 0.74 (oak), indicating a good match between the model and real data with a significant correlation at $p < 0.01$.

Table 3. Variables of the exponential decay model, and determination coefficients

Material	Model variables			R^2
	$LR_{COD}(0)$, g/m^3/d	r	θ, g/m^3/d	
Sawdust	442	0.056	14	0.98
Bark	556	0.113	78	0.89
Larch	613	0.074	3	0.79
Oak	317	0.058	9	0.74
Poplar	99	0.027	5	0.96
Spruce	150	0.063	35	0.95

COD leaching rates were initially high, varying from 613 g/m^3/d (larch) to 99 g/m^3/d (poplar). However, their decrease over time was rapid. After only 18 weeks of operation the values exhibited by the denitrifying bioreactors were almost constant. The lowest and highest asymptote rate was achieved with larch (3 g/m^3/d) and mulch (78 g/m^3/d), respectively.

Larch woodchips exhibited the highest initial COD leaching rate and at the same time the lowest asymptote rate. Based on data (COD concentrations) from the first 18 weeks of denitrifying bioreactor operation, the total amounts of COD which would be released from a volume unit of a bioreactor after 1 and 10 years were estimated (using a definite integral), (Figure 8).

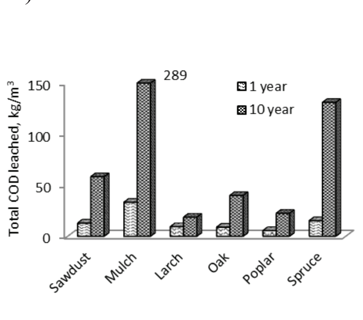


Figure 8. The total amounts of COD which would be released from a volume unit of a bioreactor after 1 and 10 years depending on the fill medium

The 1 year total COD amount increased in the following order: poplar (5.52 kg/m^3) < oak (8.88 kg/m^3) < larch (9.36 kg/m^3) < sawdust (12.9 kg/m^3) < spruce (15.3 kg/m^3) < mulch (33.3 kg/m^3). Over a long-term period the order was similar but not the same. The lowest and highest 10 years total COD amount was achieved with larch (18.7 kg/m^3) and mulch (289 kg/m^3), which corresponds with their asymptote rates. The 10 years total COD amounts which would be released from other studied denitrifying bioreactor fill media were as follows: poplar (22.5 kg/m^3) < oak (39.9 kg/m^3) < sawdust (58.4 kg/m^3) < spruce (131 kg/m^3). From the data it is obvious that total released COD amounts can be very different depending on the fill medium used.

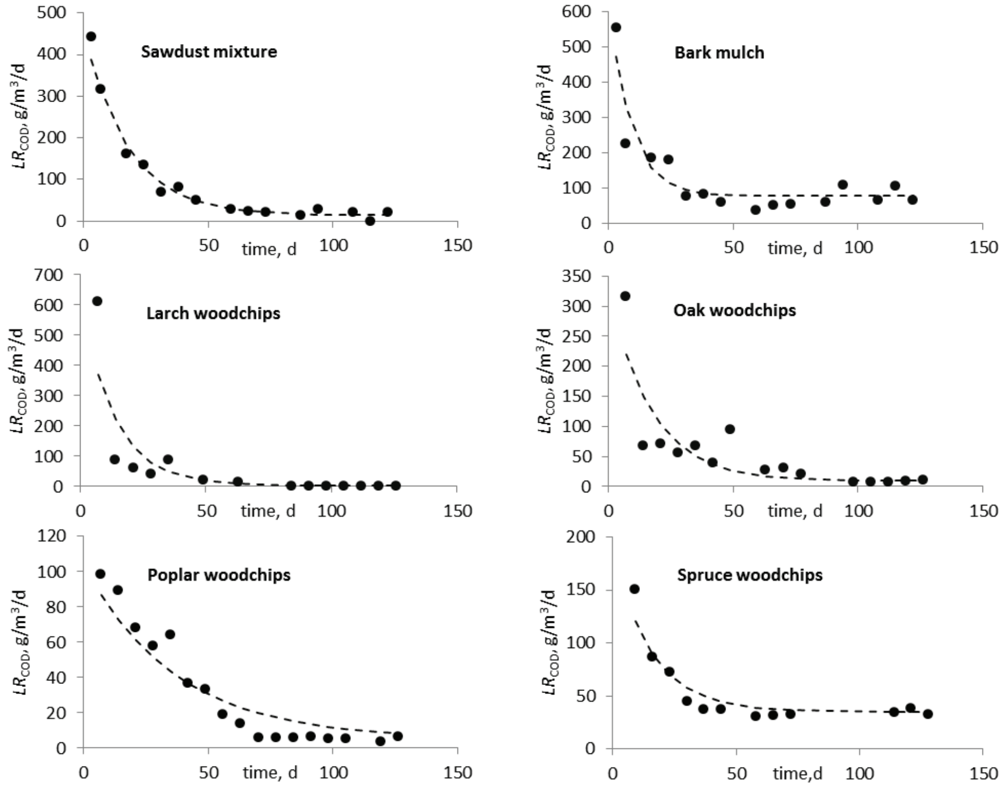


Figure 9. Decrease in COD leaching rates over time

CONCLUSIONS

The start-up phase of denitrifying bioreactors is the most critical period of their operation. During this period, denitrification rates gradually increase and organic compounds, which are necessary for denitrification, are released from the fill media in excessive amounts.

In our experiments, the denitrification rates reported for all fill media increased, but with different slopes. High inlet NO_3-N concentrations and higher temperatures led to higher denitrification rates. With respect to nitrate removal rate and efficiency, good results were achieved at the end of the start-up phase with sawdust, mulch, oak, and poplar. On the other hand, larch and spruce did not appear to be suitable carbon sources for denitrifying bioreactors.

There were differences among the concentrations and leaching rates of the released COD, BOD, and NH_4-N. However, both concentrations and leaching rates decreased substantially during the start-up phase. The best results with respect to initial

COD and BOD leaching rates were shown by poplar, although the lowest 10 years total COD amount would be achieved with larch. Leaching of NH_4-N was generally low, with the exception of sawdust. Longer HRTs and lower operating temperatures foster a slowdown in the decomposition and leaching of fill media.

Based on the results reported both for organic compound leaching and nitrate removal, poplar seems to be the most suitable denitrifying bioreactor fill medium of all the tested materials.

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