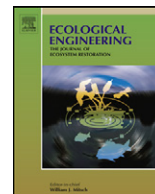




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Enhanced denitrification in a bioaugmented horizontal subsurface flow filter

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ABSTRACT

The effect of adding low concentrations of a sediment/microbial community suspension to speed up the development of the denitrification capacity in the restored HSSF of a hybrid CW was studied during a one-year period after the filling of the horizontal filter with new light weight aggregates (LWA). Two HSSF filters with the same LWA substrate but different wastewater flow regimes were used as donor systems for the bioaugmentation. NO₃-N concentrations in the outflows of all variants of studied MCs were significantly influenced by the time factor ($p < 0.001$, repeated measures ANOVA). Post hoc comparison indicated that MCs bioaugmented with the sediment suspension from a similar HSSF had significantly lower NO₃-N concentrations than the control MCs ($p < 0.05$, Fisher LSD test), whereas MCs bioaugmented with the sediment suspension from a less similar HSSF did not show significant differences compared to the control MCs. This finding emphasizes the importance of the similarity of flow regime and water parameters in choosing a donor system for bioaugmentation. High variability of the effect of bioaugmentation shows that its importance for full scale operation may be overshadowed by the effect of other factors determining denitrification intensity.

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1. Introduction

The duration of the effective functioning of constructed wetlands (CW) for wastewater treatment is limited (Kadlec and Knight, 1996). One of the most typical reasons for this is the saturation of filter materials with phosphorus (Drizo et al., 2002). Although there are many studies underway to find filter materials with longer P sorption capacity, the restoration of CWs must be performed sooner or later. In terms of subsurface flow filters, this means that the filter material has to be replaced with new, non-saturated substrates. A suitable microbial community for wastewater treatment in CWs usually develops spontaneously from the microbes that are previously present in the substrate and microbes that arrive with the inflowing wastewater (Decamp and Warren, 2001; Silyn-Roberts and Lewis, 2001; Stevik et al., 2004). In our study we used filter material that is generally referred to as *Light Weight Aggregates* (LWA). In some cases the synonym LECA (*Light Expanded Clay Aggregates*) is also used (see Brix et al., 2001). The filter material in newly restored LWA-based CWs is normally germ-free and may even have unfavorable environmental conditions for the microbes arriving with the inflowing wastewater. According to Nurk et al. (2009), the proportion of microbial organics in the overall organics in an LWA-

based horizontal subsurface flow (HSSF) CW was low: 0.4 ± 0.7 mg microbial N/kg dm, whereas the amount of carbon in the functioning HSSF CW was nearly 1000 mg C/kg dm. At the same time, HSSF CW systems based on LWA have the benefits of good water conductivity, which lowers the risk of clogging, and low heat conductivity, which makes them preferable in cold climate conditions and, in the case of high Ca and Mg content, also a high phosphorus adsorption capacity (Wittgren and Maehlum, 1997; Zhu et al., 1997, 2003).

Bioaugmentation has been reported as a suitable measure to enhance microbial activities in polluted soils (Andreoni et al., 1998) and wetlands (Simon et al., 1999). Few studies have been performed on the efficiency of bioaugmentation in treatment wetlands concerning the degradation of pesticides (Runes et al., 2001), organic chemicals (Simon et al., 2004), PAHs (Yu et al., 2005), and the removal of heavy metals (Park et al., 2008; Lampis et al., 2009). Several experiments have, however, been performed to enhance nitrogen removal. Adding a specially adapted microbial community (Rustige and Nolde, 2006), inoculation with anammox bacteria (Paredes et al., 2006) and bioaugmentation with amended soil (Zou et al., 2009) yielded positive results.

The microbial communities of different substrates have different structures, and therefore the best results in bioaugmentation have been achieved with microbial strains or microbial communities that have been isolated from the same polluted environment, because microbial communities that are isolated from the same environment are adapted to it, whereas introduced microbes will

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be outcompeted (Bento et al., 2005; Simon et al., 2004). Nevertheless, to achieve more effective purification in CWs, the use of molecular and microbial techniques is of crucial importance (Tonderski, 2010).

In our earlier study (Nurk et al., 2009) we found in a pilot-scale trial that bioaugmentation resulted in a change in the structure of the microbial community of the supplemented mesocosms (MCs) during a study period of nearly half a year. In this study we tried to prove that the use of bioaugmentation on the new substrate of LWA-based HSSF filter of a hybrid constructed wetland may have a significant positive effect on the denitrification efficiency of the filters. We also expected that bioaugmentation would have a longer-term effect on denitrification, and therefore the study period was prolonged.

We were interested in how the addition of sediment suspension, which has a low content of organics and nutrients and has been taken from an HSSF filter that has a similar substrate, influences denitrification during a one-year period after filling the horizontal filter with new LWA. We sought to discover how bioaugmentation works: (1) if the donor system has a similar substrate, flow regime and wastewater pre-treatment level to the study system, (2) when only the substrate is similar and the changes in the working regime of the donor system may have hindered denitrification of the microbial community.

2. Methods

The bioaugmentation trial was performed in the HSSF MCs of the hybrid CW experimental system that had already been used for the pilot-scale experiment described by Nurk et al. (2009). The pilot-scale trial (see Section 1) permitted us to determine the approximate amounts of sediment suspension for further trials.

2.1. Site description

The study system is located on the territory of the activated sludge wastewater treatment plant (AWP) of Nõo village in Estonia. The wastewater (domestic wastewater combined with effluents from the dairy and meat industries) is pumped into the CW before it reaches the wastewater pre-filtration grid of the AWP. The exact daily water flow is controlled by a timer-operated pump. A certain amount of wastewater is first pumped into a septic tank (2 m³). After the septic tank, water flows through the interim well to the VSSF pre-treatment filters (total area 6 m²). Pre-treated wastewater flows into the distribution box, where wastewater is divided equally between 21 parallel MC cells (for each MC cell: length – 1.5 m, width – 0.2 m, depth – 0.6 m) (Fig. 1).

The HSSF MCs were filled with LWA with a particle size of 2–4 mm. A total of 9 MCs were used for the bioaugmentation experiment with LWA. On 29th October 2009, the filters were covered with 5-cm-thick insulation slabs to avoid the freezing of the wastewater due to low temperatures. The insulation slabs were removed on 12 April 2010.

2.2. Setup of experiment

The experiment was started in the summer period in order to allow for the faster growth of microbes due to the higher water temperatures than in the previous trial, and its set-up was based on data obtained from the previously mentioned pilot-scale experiment. We assumed that lowering the wastewater retention time inside the HSSF MCs should make the differences in NO₃-N concentrations in parallel outflows more noticeable when control MCs are compared to bioaugmented MCs. The wastewater retention

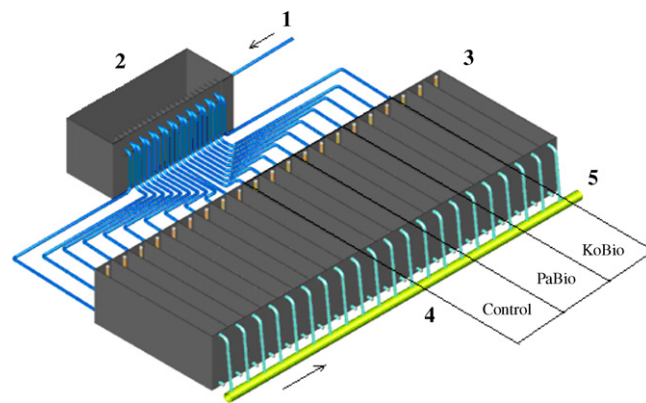


Fig. 1. The design of the HSSF part of the Nõo hybrid CW study system. (1) Inflow from the VSSF pre-treatment filter, (2) wastewater distribution box for the inflows of the HSSF mesocosms (MCs), (3) HSSF MCs, (4) outflow pipes and collector pipe, (5) outflow from the HSSF part of the Nõo study system. MCs used for the trial: control – control MCs, PaBio – MCs with microbial suspension from Paistu CW, KoBio – MCs with microbial suspension from Kodijärve CW. MCs not surrounded by a rectangle were not used in the bioaugmentation trial.

time in the HSSF MCs was lowered to 1.2 days and kept stable during the entire trial period. The area and volume of the vertical pre-treatment filter was increased to avoid overloading it. This change was introduced to ensure that wastewater flowing into the HSSF part of the hybrid CW has a similar TOC/BOD₇ ratio and NO₃-N concentrations to the beginning of the pilot-scale experiment described by Nurk et al. (2009). Mesocosm nos. 10, 11, 12 counting from the right edge of the HSSF if following the water flow direction through it were used as control MCs. Mesocosm nos. 13, 14, 15, and 16, 17, 18 were bioaugmented with the sediment suspension from Paistu and Kodijärve HSSFs respectively. Mesocosm nos. 1–9 were not used in our experiment (Fig. 1).

2.3. Production of the microbial suspension and inoculation of mesocosms

On 5 June 2009, newly established LWA-based HSSF mesocosms were inoculated with a microbial community/sediment suspension produced through the robust mechanical treatment of the substrate collected from the inflow parts of the donor systems, two different LWA-based HSSF filter beds of hybrid CWs. The first donor system treats wastewater from Paistu Basic School in Viljandi County, Estonia and has been operating since 2002 (Öövel et al., 2007). The second donor system, treating wastewater from a home for the elderly in Kodijärve, Estonia, was renovated and has been operating as a new system since 2005. The main difference between the donor systems regarding our experiment was the age and stability of the conditions for the development of a denitrifying microbial community: (1) the HSSF filter bed of Paistu hybrid CW received wastewater that had been pre-treated in a vertical subsurface flow (VSSF) filter, whereas the vertical filter of the Kodijärve hybrid CW was not in use during the six-month period before the collection of the substrate for bioaugmentation, and was in use with short occasional periods of non-usage during the two preceding years, (2) wastewater flowing into the Kodijärve hybrid CW has been re-circulated with different re-circulation regimes, whereas wastewater flowing into the Paistu hybrid CW has not been re-circulated. Therefore the Paistu HSSF filter bed was a relatively stable environment compared to the Kodijärve HSSF filter bed.

Data regarding BOD₇ and NO₃-N values in the inflows and outflows of both donor HSSF and BOD₇ values in the inflows of VSSF pre-treatment filters are indicated in Table 1. The aver-

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