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Overland flow systems for treatment of landfill leachates—Potential nitrification and structure of the ammonia-oxidising bacterial community during a growing season

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Abstract

Overland flow systems are useful for treating landfill leachates, because they provide favourable conditions for nitrification and they are easy to maintain. However, little is known about the microbial communities in such systems or the nitrification capacity of those microorganisms. In this study, seasonal variations in potential nitrification and in community composition of nitrifying bacteria were investigated in two overland flow areas receiving leachate from landfills at Korslöt and Hagby, Sweden. Samples were collected in the settling ponds sediment and at two depths in the overland flow areas (the macrophyte litter layer and the rhizosphere) in May, August and November 2003. A short-term incubation method was used to measure potential oxidation of ammonia and nitrite (designated PAO and PNO). The ammonia-oxidising bacterial (AOB) community was investigated using a 16S rRNA gene approach that included PCR amplification and analysis of PCR products by denaturing gradient gel electrophoresis (DGGE), followed by nucleotide sequencing and phylogenetic analysis.

PAO was determined in the range 5–2700 $(NO_2^- + NO_3^-)$-N g^{-1} dw d^{-1} and PNO in the range 60–2000 μg NO_2^--N g^{-1} dw d^{-1}. At Korslöt, PAO and PNO showed similar temporal variation in the different ecosystems, whereas no such relationship was noticed at Hagby. Considering both sites, there was no obvious change in the composition of the AOB community over the growing season. However, the composition did differ between the ecosystems: *Nitrosomonas*-like sequences were more common in the ponds, and in the litter layers they were found as often as *Nitrosospira*-like sequences, whereas *Nitrosospira*-like sequences were more common in the rhizospheres. Altogether, we found nine different AOB sequences, five *Nitrosomonas*-like and four *Nitrosospira*-like, which belonged to clusters 0, 2, 3b, 6a, 6b and 7. There was no apparent relationship between the number of AOB populations and the PAO in different soil layers and sediments.

Keywords: Ammonia-oxidising bacterial community; Landfill leachates; Nitrification; Overland flow; 16S rDNA; Denaturing gradient gel electrophoresis

1. Introduction

Sweden and the rest of the European Union (EU) are attempting to decrease the human-based discharge of nitrogen to the environment. Actions aimed at reducing emissions from both point and nonpoint nitrogen sources are being undertaken. One of the most noticeable problems associated with landfill leachates is the high concentration of ammonium, as exemplified by a study of 11 landfills in Sweden, which revealed levels between 93 and 870 mg NH_4^+-N l^{-1} (Öman et al., 2000). In the past, landfill leachates from settling ponds in Sweden were usually conveyed to municipal wastewater treatment plants, but in recent years the trend has been to treat the leachates in on-site systems such as aerated ponds and constructed wetlands, near the landfill (Mulamoottil et al., 1999). Sundblad (1998) and Andersson et al. (2005) have reported satisfactory nitrogen reduction in municipal sewage water treated in constructed shallow basins dominated by emergent macrophytes in Sweden. Results regarding

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nitrogen removal in free water surface wetlands, shallow basins with emergent macrophytes, have indicated that overland flow systems may be efficient in nitrifying ammonium-rich wastewater. In recent years, systems of that type have been chosen to treat landfill leachates, because they provide favourable conditions for nitrification (i.e. good oxygen availability and ample surface areas for the biofilms) and they are easy to maintain (Reed et al., 1988). Both living macrophytes and macrophyte litter are important attachment surfaces for biofilms (Flood et al., 1999), which are responsible for the majority of the microbial processes that occur in wetlands (Brix, 1994).Thompson et al. (1995) noted seasonal variations in nitrification in a saltwater wetland and indicated that they might be explained by competition for available oxygen due to oxygen consumption during heterotrophic decomposition. It has also been reported that nitrification rates are significantly decreased when the temperature was gradually dropped (Xie et al., 2003). In Sweden, the temperature in landfill leachates can vary from $+4\,^{\circ}C$ to $+25\,^{\circ}C$ during the year. The activity of the ammonia-oxidising bacterial (AOB) community can change in response to fluctuations in temperature as shown in a laboratory study (Cookson et al., 2002), and possibly also in reaction to variations in temperature along with altered availability of oxygen and organic matter. The field studies were not shown this fluctuations (Cookson et al., 2002). It is also possible that these composition of the AOB community respond to the temperature variations and changes in oxygen and organic matter availability. In general, studies of AOB in biofilms and activated sludge have shown that a single population dominates the system particularly when the ammonia availability is high. Schramm et al. (1996) studied biofilm grown on plastic foils in ammonium-rich trickling filter and detected a predominance of *Nitrosomonas europaea*-like organisms, *Nitrosomonas mobilis* was dominating in activated sludge from industrial wastewater (Juretschko et al., 1998) and *Nitrosomonas* sp. dominating in biofilm from domestic wastewater (Okabe et al., 1999). Substrate concentrations, K_m values and the ecological context, that *Nitrosospira* sp. had higher substrate affinity but lower maximum activity (*K* strategist) compared to the *r* strategist *N. europaea,* were discussed as possible explanations by Schramm et al. (1996). Similarly, Webster et al. (2002) found that the unfertilised grassland had a more diverse AOB community compared to the fertilised grassland, studied with CTO and *amoA* primers, which might reflect the physical and chemical heterogeneity of the undisturbed soils.

The aim of our study was to investigate temporal variations in the potential oxidation of ammonia and nitrite and the composition of the ammonia-oxidising bacterial community during a growing season in overland flow areas used to treat landfill leachates. We had two hypotheses; first, that the potential activity, indicating the size of the active community, will decrease in response to the temperature decrease in the end of the year. The second was that the high ammonia load in the landfill leachates will favour development of a community with one single dominating AOB population, most likely some *Nitrosomonas* sp.

Samples were collected over one growing season from two landfill leachate treatment systems located in constructed wetlands in southeast central Sweden. The potential oxidation of both ammonia and nitrite was determined by a short-term incubation method under favourable conditions. The results can be regarded as an estimate of the maximum activity and also as an indication of the size of the active nitrifying bacterial community (Schmidt and Belser, 1994). A 16S rRNA-based approach was employed to analyse the population structure and dynamics of the AOB communities.

2. Materials and methods

2.1. Description of the study sites

The study was carried out in southeast central Sweden in two landfills that contain mainly household and construction/industrial waste. For the leachate water, each landfill has a settling pond followed by an overland flow system with intermittent application (8 h watering and 16 h drying) to stimulate the nitrification, and finally a pond intended primarily for denitrification. The overland flow and wetland systems at both sites were designed by Water Revival Systems (WRS) Uppsala AB, Uppsala, Sweden, and they each have a well-defined inlet and outlet. Employees at the landfills collect water samples at designated times for nutrient analyses.

Korslöt recycling site in Vagnhärad is run by Trosabygdens Teknik AB. The $8000\,m^2$ overland flow area at Korslöt is located on old farm land and was sowed with *Phalaris arundinacea* (L.). In 2003, the area contained a fairly well mixed community of *P. arundinacea* and *Phragmites australis* (Cav.) that had invaded the area by seeds. Part of the area ($800\,m^2$) has been irrigated with landfill leachate (mean conc. $136\,mg\,NH_4^+\text{-}N\,l^{-1}$, $600\,m^3\,month^{-1}$) since January 2003, and it received municipal wastewater in 2000–2002. The water is applied through an open channel that is continuously flooded, and the system is in operation throughout winter. The overland flow area is harvested annually in October.

Hagby recycling site in Täby is run by Söderhalls Renhållningsverk AB. The $11{,}600\,m^2$ overland flow area at Hagby is situated on organic soil. It was constructed in 2002 to treat all the landfill leachate (mean conc. 78 mg $NH_4^+\text{-}N\,l^{-1}$, $15{,}000\,m^3\,month^{-1}$) and was sowed with a mixture of *P. arundinacea* and *Agrostis stolonifera* (L.). In 2003, the area was dominated by *P. arundinacea* with *A. stolonifera* growing in small patches between the *P. arundinacea* shoots. The water is applied through gated pipes, and the system is in operation only during the growing season from April to November.