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Investigation of Archaeal and Bacterial community structure of five different small drinking water networks with special regard to the nitrifying microorganisms

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ABSTRACT

Total microbial community structure, and particularly nitrifying communities inhabiting five different small drinking water networks characterized with different water physical and chemical parameters was investigated, using cultivation-based methods and sequence aided Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis. Ammonium ion, originated from well water, was only partially oxidized via nitrite to nitrate in the drinking water distribution systems. Nitrification occurred at low ammonium ion concentration (27–46 μM), relatively high pH (7.6–8.2) and over a wide range of dissolved oxygen concentrations (0.4–9.0 mg L^{-1}). The nitrifying communities of the distribution systems were characterized by variable most probable numbers (2×10^2–7.1×10^4 MPN L^{-1}) and probably originated from the non-treated well water. The sequence aided T-RFLP method revealed that ammonia-oxidizing microorganisms and nitrite-oxidizing Bacteria (*Nitrosomonas oligotropha*, *Nitrosopumilus maritimus,* and *Nitrospira moscoviensis,* '*Candidatus* Nitrospira defluvii') were present in different ratios in the total microbial communities of the distinct parts of the water network systems. The nitrate generated by nitrification was partly utilized by nitrate-reducing (and denitrifying) Bacteria, present in low MPN and characterized by sequence aided T-RFLP as *Comamonas* sp. and *Pseudomonas* spp. Different environmental factors, like pH, chemical oxygen demand, calculated total inorganic nitrogen content (moreover nitrite and nitrate concentration), temperature had important effect on the total bacterial and archaeal community distribution.

1. Introduction

In Hungary 70% of drinking water originates from underground sources. The physical (temperature, colour), and chemical (dissolved oxygen [DO], pH, NH_4^+, NO_2^-, NO_3^-, chemical oxygen demand [COD] etc.) parameters of water and the applied water treatments have deep influence on the biological processes taking place in drinking water networks (Odell et al., 1996). Ammonia, for example present in well water, can be biologically oxidized to nitrate via nitrite by the process of nitrification. At first, ammonia-oxidizing Bacteria (AOB) oxidize ammonia to nitrite. Subsequently, nitrite-oxidizing Bacteria (NOB) oxidize the generated nitrite to nitrate. In addition to AOB, ammonia-oxidizers belonging to the domain Archaea play important role in autotrophic ammonia oxidizing process. Ammonia-oxidizing Archaea (AOA) have been detected mainly by cultivation independent molecular methods in marine water, sediments, soils, and wastewater (Park et al., 2010). However, few studies examined the occurrence of AOA in drinking water. Granular activated carbon can be colonized by AOA dominated ammonia-oxidizing microbial community (Kasuga et al., 2010) and the number of AOA could exceed the number of AOB in drinking water (van der Wielen et al., 2009).

The prevailing conditions in drinking water could be appropriate for nitrification process (Lipponen et al., 2002; van der Wielen et al., 2009). However, the optimal environmental parameters for nitrifying microorganisms show differences. Ammonia-oxidizing prokaryotes can tolerate low temperature (<12 °C) (Pintar and Slawson, 2003), low pH [AOB: pH > 6.5 (Tarre and Green, 2004;) and AOA: pH > 2.5 (Hatzenpichler, 2012; Stahl and de la Torre, 2012)], and low DO [AOA: <3.1 μM-0.2 mM (Erguder et al., 2009) and AOB can tolerate even anoxic conditions (Zhang et al., 2009)]. Ammonia concentration (Martens-Habbena et al., 2009; Hatzenpichler, 2012) and temperature (Urakawa et al., 2008; Junier et al., 2010) are considered important parameters affecting the growth and diversity of

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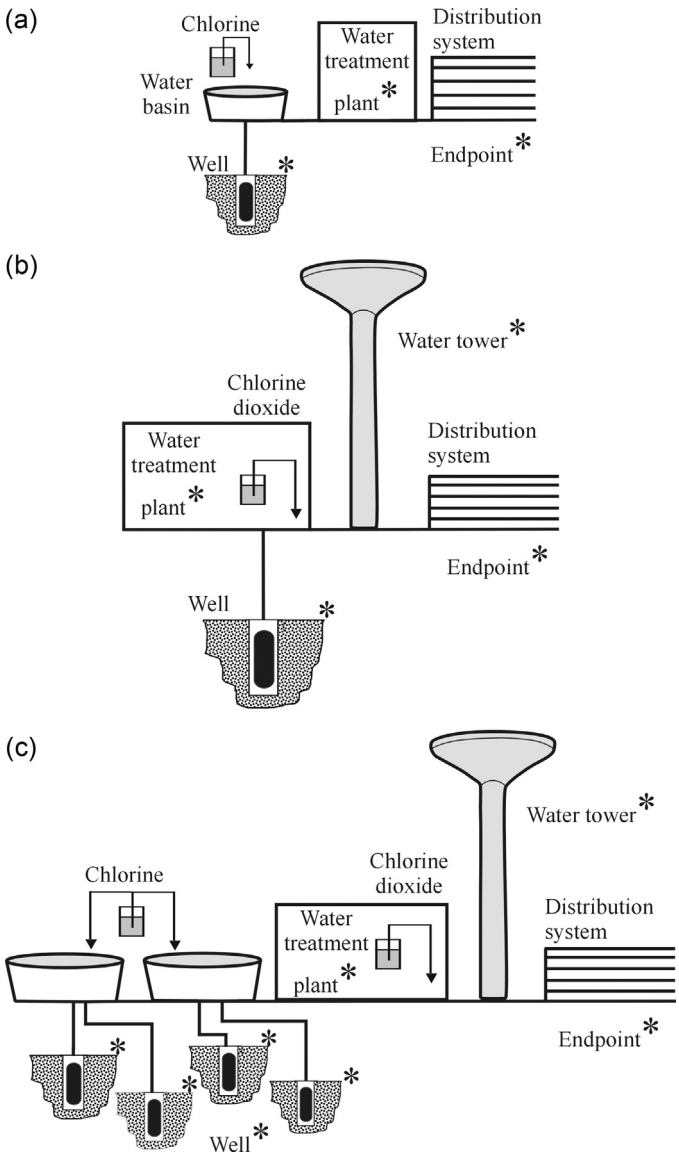

Fig. 1. Schematic construction of the examined water networks (WN). The location of disinfectant dosage and sampling points (*) are indicated in Figs. 1a: The schematic construction of WN-1. 1b: The construction of WN-2, WN-3 and WN-4 are similar, as presented in Fig. 1c: The schematic construction of WN-5.

AOB and AOA. AOA were found to be more diverse at temperatures around 19 °C in aquarium biofilters (Urakawa et al., 2008) and engineered systems (You et al., 2009). In addition, the growth of AOA could be facilitated by long residence time (You et al., 2009; Jin et al., 2010). NOB are more sensitive to the fluctuation of environmental parameters than AOB, causing occasionally nitrite accumulation in drinking water.

Growth and activity of nitrifying microorganisms are greatly influenced by the structure and operating parameters of drinking water systems and the applied disinfection processes (Odell et al., 1996). The most common disinfection processes are chlorination (using chlorine gas, hypochlorite), chloramination, chlorine dioxide dosage, ozonization, and UV irradiation. Chlorine is a powerful oxidant, although its concentration reduces rapidly by oxidizing EPS matrix of the biofilm. On the other hand, chloramines could be generated due to the reaction of chlorine and ammonium ions. However, chloramines are used as disinfectants owing to their stability and ability to penetrate into biofilm (Morató et al., 2003). One of the most powerful disinfectants is chlorine dioxide due to its easy penetration through cell walls. It can also be used for taste and odour control via iron and manganese oxidation and no reaction with ammonia is observed (Rav-Acha, 1983). Due to the decreasing efficiency of disinfectants with time, microbial growth and thus nitrification mainly occur in the distal parts of distribution systems. There is little information about the effect of chlorine dioxide on nitrifying microorganisms, but its by-product chlorite is likely to suppress nitrification (McGuire et al., 1999).

The aim of this study was to provide a comprehensive microbiological survey of five different small drinking water networks which showed signs of nitrification episodes based on determination of inorganic nitrogen forms. Our study focused on the crucial points of the drinking water networks (well water, water treatment plant, water tower and distribution system end points) having a decisive role in water quality. The main goal was to detect, identify and quantify nitrifying microorganisms inhabiting the selected drinking water networks. The community structure of Bacteria and Archaea was explored by sequence aided Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis of 16S rRNA genes. Cultivation independent methods based on bacterial and archaeal ammonia monooxygenase A (*amoA*) and *Nitrospira* specific 16S rRNA gene based assays were used to detect nitrifying microorganisms.

2. Material and methods

2.1. Sample sites and collection

Five different small drinking water networks (WN-1 to WN-5) located in County Fejér (Hungary) were examined. Samples were collected during September and October. The ammonium ion content of well water was not removed during water treatment. In WN-1 chlorine and in three other water networks (WN-2, WN-3, WN-4) chlorine dioxide were applied as sole disinfectant. In water network WN-5, both chlorine and chlorine dioxide were applied. Chlorine was added to raw water prior to storage in water basin and chlorine dioxide was dosed to drinking water leaving water treatment plant. In water networks WN-4 and WN-5, iron and manganese removal was performed by applying potassium permanganate. The basic characteristics of the investigated water networks are summarized in Table 1 and their schematic structures are presented in Fig. 1.

Raw water samples were collected from wells (well water – W, the four wells of WN-5 are referred with a-d characters). Drinking water samples were collected at assigned sampling points of water treatment plants (Ww), and/or after water towers (Wt), and from different endpoints (Ep, the two endpoints of WN-5 are referred with a-b characters) of the distribution system (tap water). At each point following flushing for 5 min, 1 and 2 L water samples were collected aseptically. 1 L water samples were used for cultivation dependent investigations (viable colony forming unit values, nitrifying and denitrifying most probable number technique), and 2 L water samples were concentrated for genomic DNA extraction. Moreover, pH and dissolved oxygen were checked on site with a Multi 350i multimeter (WTW, Weilheim, Germany) and samples were taken for laboratory chemical analysis.

2.2. Chemical analysis

Routine water chemistry measurements were performed by the accredited laboratory of Fejérvíz Waterworks Ltd. Concentration of inorganic nitrogen forms (ammonium ion, nitrite, nitrate), chemical oxygen demand (COD), iron and chlorite concentrations were measured according to Hungarian standard methods, as follows: NH_4^+ MSZ EN ISO 11732:2005, NO_2^-, and NO_3^- MSZ 1484-13:2009,