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Unraveling the microbial community of a cold groundwater catchment system

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ABSTRACT

The abundance, diversity and composition of bacterial communities in water wells with low groundwater temperatures were assessed. The drinking water catchment system, equipped with subsurface groundwater treatment for iron- and manganese removal, is located within a continental influenced veldt landscape type in eastern Russia, close to the border to China. In this study, the bacterial communities in 22 different water wells of the catchment system were analyzed and correlated to operating conditions and environmental factors. The investigated bacterial treated and groundwater populations differed from those in central European groundwater. Large variations between the investigated samples were observed, and DGGE profiles of water samples from the beginning and the end of the abstraction phases revealed two distinct fingerprint clusters with about 82% similarity to each other corresponding to the operation mode of the wells. Sequence data analysis from 454 pyrosequencing indicated Rhodoferax and Gallionella as the most abundant genera within the catchment system. The abundance of the OTU Methylotenera was statistically significant when correlated to the beginning of the abstraction phases, while no indicator OTUs could be determined for the end of the pumping phases. ACK-M1 cluster was proofed as indicator OTU for operating wells, whereas the Gallionella OTUs were correlated with non operating wells. Well operation and resultant oxygen entry could serve as factors that altered the bacterial community structure and composition the most. Quantitative PCR analysis showed that genes related to the iron-reducing Rhodoferax genus were present in nearly all of the samples. This study clearly showed an alteration within the bacterial communities dependent on the operation mode of the water wells.

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1. Introduction

Drinking water sources include both surface water (lakes, rivers or reservoirs) and groundwater, and the latter source is of great importance in many areas. Some groundwater from anoxic aquifers contains excessive levels of iron, manganese and/or nitrogen, and treatment is often required to remove these elements prior to water use. The removal of iron and manganese from groundwater can be performed either ex-situ or in-situ. Subsurface iron removal has been applied in Europe as an established method for in situ removal of iron and manganese from groundwater (Rott et al., 2002; van Halem et al., 2010). Such treatment typically requires periodic

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injection of aerated water which creates an oxidized zone around the injection wells (Hallberg and Martinell, 1976), allowing iron and manganese oxidation (van Halem et al., 2011, 2010), primarily driven by so called iron oxidizing bacteria (FeOB). This biologically mediated Fe- and Mn- oxidation results in precipitation of insoluble oxyhydroxides, which remain in the subsurface, allowing iron- and manganese-free water to be abstracted from production wells. However, this method is hampered by clogging of the aquifer (Appelo et al., 1999; Braester, 1988) or by well screen clogging (Houben, 2006) if a single well is used for abstraction and injection of water. Furthermore, Emerson and de Vet (2015) reported the occurrence of well clogging as a result of intermitted well operation when Mn and Fe containing anaerobic groundwater is mixed with aerated water. Therefore, the worldwide abstraction of groundwater is often reduced by significantly lowered well yields. The prevention and remediation of well clogging has been investigated in many studies (Houben and Treskatis, 2007; Houben, 2006; van







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Beek et al., 2009), but microbiological colmation of the wells was not considered in these previous reports.

Colmation, as indicated by the reduction of the hydraulic conductivity of porous media, is a physical, chemical and, mainly, a microbiological process. Microbiological colmation is caused by biofilms, matrix-enclosed, microbial populations that are ubiquitous at environmental interfaces. Within the biofilm, extra-cellular polymeric substances (EPS), sheaths, stalks, and sometimes iron and manganese oxides, and organic and inorganic debris are present (Howsam, 1988). This EPS serves as the habitat for the bacterial community and protects it against predators, UV light and disinfection. Injection of aerated water through infiltration wells causes a shift from anaerobic to aerobic conditions in the aquifer. These changes can promote growth of microbial biofilms and increased Fe and Mn precipitation (Bourg and Bertin, 1993; Hiscock and Grischek, 2002). These biofilms can form at water-solid interfaces within the well system and can ultimately result in complete clogging of pipes, screen slots, and pumps, as well as of interstitial pore spaces in the aquifer sediments (Flemming, 2002; Howsam, 1988).

To overcome the problem of biological clogging of water abstraction systems, a greater and more detailed knowledge of the bacteria involved in biofilm formation is required. Bacteria frequently found in iron-related, well clogging situations are *Gallionella ferruginea* (Vet et al., 2012), *Leptothrix ochracea* (Hallbeck et al., 1993), *Sphaerotilus natans* (Gino et al., 2010), *Crenothrix polyspora* (Stoecker et al., 2006), *Clonothrix fusca* (Vigliotta et al., 2007) and also members of the genus *Pseudomonas* (Liu et al., 2014). Furthermore, well clogging in anaerobic zones can also be caused by sulfate-reducing bacteria (SRB) (Shi et al., 2016; Van Beek and Van der Kooij, 1982).

Numerous analyses of microbial community structure in natural (Blöthe and Roden, 2009; Flores-Rentería et al., 2016; Ionescu et al., 2012; Wu et al., 2016) and engineered water systems (Berry et al., 2006; Cerrato et al., 2010; Douterelo et al., 2014; Revetta et al., 2016) have been reported in the literature over the last several decades. In many studies, molecular microbial fingerprint techniques like denaturing gradient gel electrophoresis (DGGE) were used for the comparison of microbial biofilm consortia (Braun et al., 2006; Eichler et al., 2006). Nevertheless, studies investigating the entire bacterial community in groundwater and abstraction wells have rarely been conducted. To obtain more

detailed information on microbial consortia, cloning and sequencing of the 16S rRNA gene is a common method (Chouari et al., 2010; Kato et al., 2009). More recently, high-throughput sequencing methods, e.g., 454 pyrosequencing, have been used to study microbial diversity in groundwater and biofilm community structure in water systems (Liu et al., 2014; Pinto et al., 2012). Most previous studies examined mesophilic biofilm communities in water systems with circumneutral pH. Several high-throughput sequencing investigations have also studied biofilm communities in acidic environments (Edwards et al., 2006; Wang et al., 2014). However, no studies have compared cold-adapted, microbial populations in untreated, low-temperature groundwater to those in groundwater altered by water abstraction or after in situ iron- and manganese removal. Since drinking water treatment plants also operate in areas with low groundwater temperatures, it is critical to also obtain insights into bacterial populations and community structures in such habitats.

In this study, the phylogenetic diversity and relative abundance of bacteria in iron rich- and de-ironed groundwater samples were assessed. The identification of parameters which control community structure can potentially be of assistance in the prevention and remediation of the colmation of the groundwater catchment. Samples from infiltration water, abstraction water and different control points (non-operating wells that were equivalent to natural groundwater) were examined to develop a comprehensive picture of the bacterial community composition and to identify abundant bacterial groups. The use of species-specific, quantitative real-time PCR (gPCR) enabled identification of relevant changes within the water wells. The specific qPCR was utilized to monitor the following bacterial groups: (i) total bacteria, (ii) Rhodoferax sp., (iii) Crenothrix polyspora, and (iv) Gallionella sp. Additionally, 16S rDNA-based techniques as the PCR-DGGE, supported by 454 pyrosequencing, were used to observe alterations in the bacterial community composition that resulted from well operation.

2. Materials and methods

2.1. Sampling site

The site is located near Khabarovsk in eastern Russia close to the Chinese border at the Amur River (Fig. 1). Samples derived from 22 wells in four different well-clusters that commenced operation at

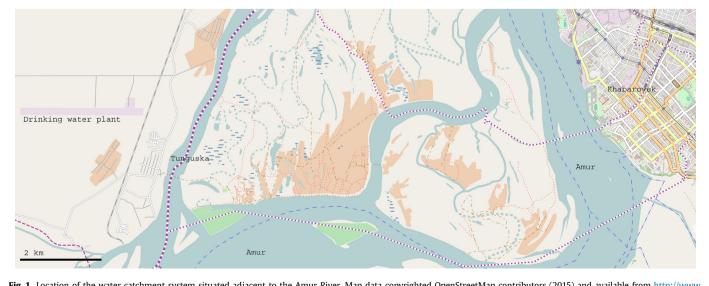


Fig. 1. Location of the water catchment system situated adjacent to the Amur River. Map data copyrighted OpenStreetMap contributors (2015) and available from http://www.openstreetmap.org.

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