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Revealing the microbial community structure of clogging materials in dewatering wells differing in physico-chemical parameters in an open-cast mining area

Juanjuan Wang [a], Maren Sickinger [a], Valerian Ciobota [b], Martina Herrmann [a,e], Helfried Rasch [c], Petra Rösch [b], Jürgen Popp [b,d], Kirsten Küsel [a,e,*]

[a] Aquatic Geomicrobiology, Friedrich Schiller University Jena, Dornburgerstr. 159, 07743 Jena, Germany
[b] Institute of Physical Chemistry, Friedrich Schiller University Jena, Helmholtzweg 4, 07743 Jena, Germany
[c] Vattenfall Europe Mining AG, Vom-Stein-Straße 39, 03050 Cottbus, Germany
[d] Institute of Photonic Technology Jena, Albert-Einstein-Straße 9, 07745 Jena, Germany
[e] German Center for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, 04103 Leipzig, Germany

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ABSTRACT

Iron rich deposits cause clogging the pumps and pipes of dewatering wells in open-cast mines, interfering with their function; however, little is known about either the microbial community structure or their potential role in the formation of these deposits. The microbial diversity and abundance of iron-oxidizing and -reducing bacteria were compared in pipe deposit samples with different levels of encrustation from 16 wells at three lignite mining sites. The groundwater varied in pH values from slightly acidic (4.5) to neutral (7.3), Fe(II) concentrations from 0.48 to 7.55 mM, oxygen content from 1.8 to 5.8 mg L^{-1}, and dissolved organic carbon (DOC) from 1.43 to 12.59 mg L^{-1}. There were high numbers of bacterial 16S rRNA gene copies in deposits, up to 2.5×10^{10} copies g^{-1} wet weight. Pyrosequencing analysis of bacterial 16S rRNA genes revealed that *Proteobacteria* was the most abundant phylum (63.3% of the total reads on average), followed by *Actinobacteria* (10.2%) and *Chloroflexi* (6.4%). *Gallionella*-related sequences dominated the bacterial community of pipe deposits and accounted for 48% of total sequence reads. Pipe deposits with amorphous ferrihydrite and schwertmannite mostly contained *Gallionella* (up to 1.51×10^{10} 16S rRNA gene copies g^{-1} wet weight), while more crystalline deposits showed a higher bacterial diversity. Surprisingly, the abundance of *Gallionella* was not correlated with groundwater pH, oxygen, or DOC content. *Sideroxydans*-related 16S rRNA gene copy numbers were one order of magnitude less than *Gallionella*, followed by acidophilic *Ferrovum*-related groups. Iron reducing bacteria were detected at rather low abundance, as was expected given the low iron reduction potential, although they could be stimulated by lactate amendment. The overall high abundance of *Gallionella* suggests that microbes may make major contributions to pipe deposit formation irrespective of the water geochemistry. Their iron

* *Corresponding author*. Friedrich Schiller University Jena, Institute of Ecology, Aquatic Geomicrobiology, Dornburgerstr. 159, D-07743 Jena, Germany. Tel.: +49 3641 949461; fax: +49 3641 949402.
E-mail address: kirsten.kuesel@uni-jena.de (K. Küsel).
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oxidation activity might initiate the formation of amorphous iron oxides, potentially providing niches for other microorganisms later after crystallization, and leading to higher bacterial diversity along with deposit accumulation in later stages of clogging.

1. Introduction

Well clogging is a well-known problem that occurs at large geographical scales (Houben and Weihe, 2010; Vanbeek, 1989; Weidner et al., 2012). It leads to reduced water permeability and a decline in well performance, and requires expensive rehabilitation and prevention practices. The clogging process can happen extremely fast, occurring sometimes within months after replacement of the pumps (Ralph and Stevenson, 1995) as the pipes and pumps are incrusted with pipe deposits and corrosion resulting from the oxidation and precipitation of iron and manganese oxides (Houben, 2003; Tuhela et al., 1997; Vanbeek, 1989). Dewatering wells, which lower groundwater levels for mining, are particularly prone to clogging as the well screen/pipes often dry out, leading to more air entering the groundwater (Weidner et al., 2012). This may favor both chemical and biological iron oxidation. Microorganisms coprecipitate iron oxides to their negatively charged cell surface or to exopolysaccharides (EPS) (Caldwell and Caldwell, 2004; Ghiorse, 1984; Konhauser, 1998), which can then act as a sorbent for organic carbon, a potential substrate for chemoorganotrophic microorganisms (Ridgway et al., 1981), which, in turn, act as locus for iron oxides and thus accelerate their deposition.

Other microorganisms, like iron-oxidizing bacteria (FeOB), can actively catalyze the oxidation of ferrous [Fe(II)] to ferric [Fe(III)] iron, the first step in the formation of iron-oxides (Larroque and Franceschi, 2011; Taylor et al., 1997). However, the importance of microbial versus chemical iron oxidation is still a matter of debate. Under acidic conditions, iron oxidation is driven exclusively by microbial activity (Baker and Banfield, 2003; Hedrich et al., 2011) as Fe(II) is stable at low pH ($pH < 4$), while at slightly acidic to circumneutral pH, FeOB compete with rapid chemical oxidation, especially under fully aerated conditions (Sung and Morgan, 1980). Therefore, neutrophilic FeOB often prefer micro-oxic conditions, where they can successfully compete with abiotic iron oxidation (Neubauer et al., 2002; Rentz et al., 2007; Sobolev and Roden, 2002). Neutrophilic iron oxidizers such as *Gallionella* are commonly associated (among others) with iron precipitation and their presence has been suggested as evidence for biological iron oxidation (Fru et al., 2012). As microaerophiles, they prefer oxygen gradients such as those that might occur in wells, especially where water from anoxic aquifers rich in Fe(II) meets oxygen introduced by pumping (Taylor et al., 1997). Recently it was shown that *Gallionella* spp. can also grow in fully aerated groundwater in labscale reactors and in trickling filters between pH 6.5 to 7.7 where biological oxidation appeared to be the dominant process (de Vet et al., 2011, 2012). The authors proposed that the autocatalytic chemical oxidation of iron was inhibited by adsorption of natural organic matter on iron oxide surfaces. Accordingly, a high load of DOC in groundwater should also favor microbial iron oxidation.

Clogging may also cause hygiene problems due to the accumulation of pathogenic organisms in iron oxides (Berry et al., 2006). In order to provide recommendations for efficient rehabilitation or clogging prevention, it is crucial to know whether the formation of pipe deposits is predominantly mediated by biotic or abiotic processes, as the iron oxides formed may differ in size and mineral composition (Luedecke et al., 2010). The geochemical conditions within the wells, such as pH, oxygen, and DOC may influence the microbial community structure as well as the formation of the pipe deposit materials. Under anoxic conditions in a pipe deposit, Fe(III)-reducing bacteria (FeRB) could reductively dissolve iron oxides by using ferric iron as terminal electron acceptor (Lovley, 2006; Weber et al., 2006). Consequently, formation and reductive dissolution of iron oxides could potentially be manipulated by altering microbial activity in the wells. A few previous studies have identified bacteria in pipe deposits from clogged wells using cultivation methods and confirmed the presence of FeOB, FeRB and sulfate reducers (Basso et al., 2005; Taylor et al., 1997; Vanbeek and Vanderkooij, 1982). However, there has been no attempt to correlate chemical and physical parameters with the microbial diversity and the quantification of different bacterial groups directly involved in iron pipe deposit formation and dissolution.

In the Lausitzer area in Eastern Germany, opencast lignite mining sites are being operated by the company Vattenfall. Around 500 wells were operated to lower the groundwater level and the malfunction of pumps caused by encrustation is a major problem, resulting in reduced pump life, which can be less than two months. Groundwater samples obtained from the dewatering wells studied here covered a broad pH range (4.5–7.3) and differed in their physical and chemical characteristics which suggested potential differences in both the incrustation level and the microbial community structure in the iron pipe deposits. We hypothesize that high levels of encrustation in dewatering wells are associated with a high fraction of FeOB, and that the community composition of FeOB is affected by groundwater geochemistry such as pH, DOC and oxygen contents. By comparing the microbial community composition with the biogeochemistry and mineralogy of groundwater and pipe deposits, we aim to gain more insight into the clogging mechanisms in dewatering wells.