Biofouling of reverse osmosis membranes in a process water treatment system in a gold mine

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Abstract Bacterial biofilms commonly cause biofouling in reverse osmosis (RO) membranes. The biofilm confers a protective environment to the bacteria, improving their tolerance against stress factors. In this study, bacterial biofouling was studied in a full-scale RO system, producing process water for a Finnish gold mine. Raw water bacterial levels corresponded to levels in natural waters. During normal RO operation, bacteria attached to the membrane did not detach to the concentrate water. In contrast, during washing, a high bacterial disperse from the membrane biofilm was observed. Bacterial community analysis suggests a dominant bacterium to be a biofouling indicator in this RO system.

Key words biofilm, mine water treatment, reverse osmosis

Introduction

Reverse osmosis (RO) and nano filtration (NF) are emerging techniques in mining for treatment of waste waters and recycling of process waters (Acheampong 2010). Nevertheless, fouling is a typical problem in RO systems. Salt precipitation, mainly of $CaSO_4$ and $CaCO_2$, on RO membranes is commonly considered as a fouling mechanism. Various water analyses and modelling software are used to estimate and avoid inorganic scaling conditions. Antiscalant, a chemical that prevents calcium precipitation, may also be added to the feed water to prevent the effects of scaling (Jiang 2017).

Biofouling differs from calcium precipitation. The risk of biofouling cannot be exactly calculated. It is mainly caused by bacteria which multiply, implying that a single bacterium is able to cause biofouling. The RO membrane is a favourable surface for bacterial growth; first, nutrient concentration is higher near the membrane due to concentration polarization. Second, water flow in the spiral membrane module is laminar and thus no high shear forces occur. Third, water flow continuously brings new nutrients for the bacteria to feed on. Antiscalants are usually based on phosphorous, which is often the limiting nutrient for bacteria in waters (Vrouwenvelder 2000).

Bacteria form slimy, complex communities called biofilms, onto surfaces. The slime composes of extracellular polymeric substances (EPS) excreted by the bacteria. EPS consists predominantly of extracellular polysaccharides, and to a lesser extent of proteins, lipids, and DNA (Flemming 2010). The cell density is high in biofilms, in which the bacteria are covered by EPS. Bacteria tolerate various stress factors substantially better in biofilms than as planktonic, free-swimming cells. For instance, bacteria living in biofilms may withstand biocides, toxic compounds, nutrient deprivation, as well as fluctuation in temperature and pH, among others (Garrett 2008).

Bacteria form biofilms onto all surfaces. Undesirable biofilm growth may cause damage. The biofilm formed in RO or NF membranes is called biofouling. Biofouling of the RO membrane may result in decrease in the pressure on the membrane, reduced flux and low-quality water. This usually leads to frequent need of membrane cleaning and changing as well as biocide use. A disadvantage of RO membranes is that they do not tolerate oxidising biocides (Jiang 2017).

In this study, biofouling is examined in a full-scale RO-system which produces process water for mine production. Raw water is taken from a neutralizing pond water. Prior to the RO system, the water is passed through sand filtration and a 5 µm dead-end mega filtration. The system comprises of two RO units in parallel, and only RO2 contains UV treatment. Biofilms have caused problems in both RO units. The aim of this study was to examine biofouling indicators in RO waters during membrane changing, when biofilm sample collection can be performed.

Methods

Water samples

Water samples were collected in January 2017. 50 ml of various water samples were filtered through a 0.45 µm polycarbonate filter. Bacterial cells on the filter were lysed by mechanical and chemical treatments, and the bacterial DNA was extracted using a phenol:chloroform extraction protocol followed by a sodium acetate-isopropanol precipitation and ethanol wash. The DNA was analysed as described below.

Biofilm sample

The biofilm sample was collected in November 2016 during membrane changing (fig. 1). The RO membranes were opened and the biofilm sample was collected directly from the membrane representing a determined surface area.

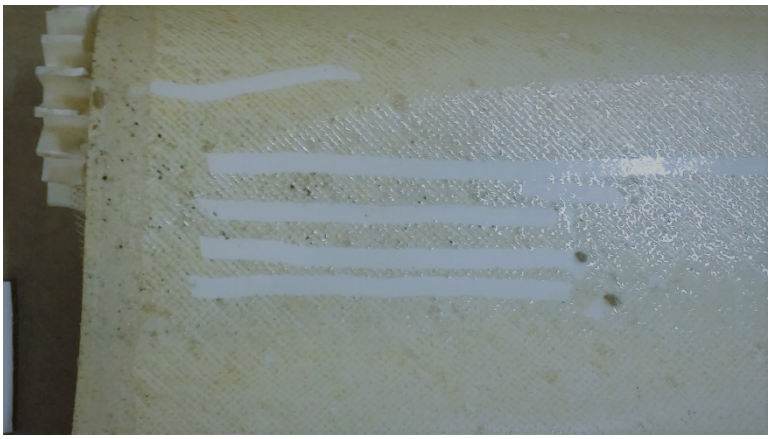

Figure 1*. Biofilm on the RO membrane. White marks represent sampling areas where biofilm was scratched, revealing the membrane.*

Total bacterial levels were measured by quantitative polymerase chain reaction (qPCR) using broad range primers developed by Nadkarni *et al.* (2002). The bacterial community was studied by next-generation sequencing (NGS) covering the variable V3-V4 region of the 16S rRNA gene (StarSEQ GmbH). In addition, the biofilm samples were sequenced from V1-V8 covering the entire 16S gene (GATC Biotech). The NGS data was processed with the USEARCH program. A 97 % similarity levels for operational taxonomic units (OTUs) were used for clustering by the UPARSE-OTU algorithm (Edgar 2013). Identification of the OTUs was based on the RDB pipeline classification (Wang *et al.* 2007).

Results

Raw water quality is presented in Table 1. Raw water have huge amount of nitrogen. Both RO system use antiscalant containing phosphate. Phosphorus in raw water remained below detection, but RO concentrate had 1 mg/L phosphorus. Before the antiscalant feed phosphorus is a limiting nutrient and after the antiscalant feed, carbon (TOC) is the limiting nutrient.

Table 1 *Nutrients in raw water.*

pH	8.4
TOC	12 mg/L
NO_3-N	10 mg/L
NH_4-N	20 mg/L
P	<0.02 mg/L

Total bacterial levels measured by qPCR broad range primers are presented in figure 2. The bacterial levels of the raw water corresponded to typical bacterial concentrations in Finnish lakes and rivers. Sand filtration decreased bacterial levels below $1{\cdot}10^6$ cells/ml. Mega filtration did not affect the concentration of bacteria. No change in the bacterial levels was observed in the pipe between the mega filter and the RO1 inlet, while the bacterial concentration decreased below $3{\cdot}10^4$ cells/ml in the RO2 water line due to UV treatment.

When operating with a 50 % recovery, bacterial levels should multiply by factor of 2 from RO inlets to RO concentrates. As bacteria levels increased only by factor of 2, bacteria attached to the membrane did not detach from the membrane to the concentrate water during normal RO operation. In contrast, after permeate reverse direction cleaning, bacterial levels reached almost $1{\cdot}10^8$ cells/ml, which implies that high concentrations of bacteria dispersed from the RO membrane biofilm to the permeate water. In the permeate water before reverse direction cleaning bacterial levels remained below $1{\cdot}10^4$ cells/ml.

Figure 3 shows the bacterial community analysis by NGS of the waters and biofilms of the RO-system. The raw water community was dominated by four bacterial genera: *Lutibacter* within *Flavobacteria, Thiobacillus* within *β-proteobacteria* and *Azotobacter* and *Pseudomonas* within *γ-proteobacteria*. *Flavobacterium* within *Flavobacteria* was detected in raw water at a 0.03 % proportion. The filtrations and RO membrane did not significantly

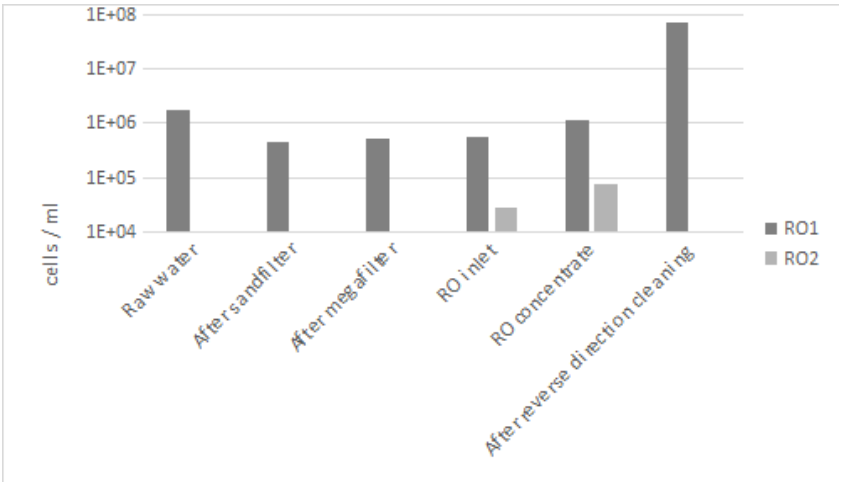

***Figure 2**. Total bacterial levels in the RO-system waters was measured by qPCR using broad range primers. The raw water and filtered water are passed to both of the ROs. Only after the megafilter, the two water lines are separated. At the RO2 water line, water is UV treated before the inlet.*

change the bacterial community. The proportions of *Lutibacter*, *Thiobacillus* and *Azotobacter* tended to decrease, suggesting that these three bacteria originating from the raw water might not be able to grow in the water treatment system. In RO1 concentrate, the proportion of *Flavobacterium* slightly increased, indicating that *Flavobacterium* may be able to grow on the membrane.

The bacterial community of the reverse direction cleaning water differed from the waters during normal RO operation. Bacterial levels in the washing water were high, indicating that bacteria from biofilms detached from the membrane to the reverse direction cleaning water during the reverse direction cleaning. Based on the bacterial community in the reverse direction cleaning water, the dominant bacterial genus in membrane biofilm is *Pseudomonas* within *γ-proteobacteria*.

The bacterial community in the biofilm, which was sampled two months earlier than the water samples, supports the hypothesis that *Pseudomonas* is a dominant bacterial genus in biofilms, as the biofilm community was dominated by two bacterial genera: *Pseudomonas* and *Flavobacterium*. For this sample, NGS sequencing covering the whole 16S rRNA gene (V1-V8 region) was available, allowing the species level identification of the major species to *Pseudomonas putida* and *Flavobacterium ahnfeltiae*.

Conclusions

Bacterial levels in RO concentrates increased from the levels in RO inlets by the same factor as water was concentrated. This implies that during normal RO operation, bacteria attached to RO biofilms do not detach from the membrane to the RO concentration water. In contrast, during reverse direction cleaning of the RO membranes, bacteria in biofilms detach to

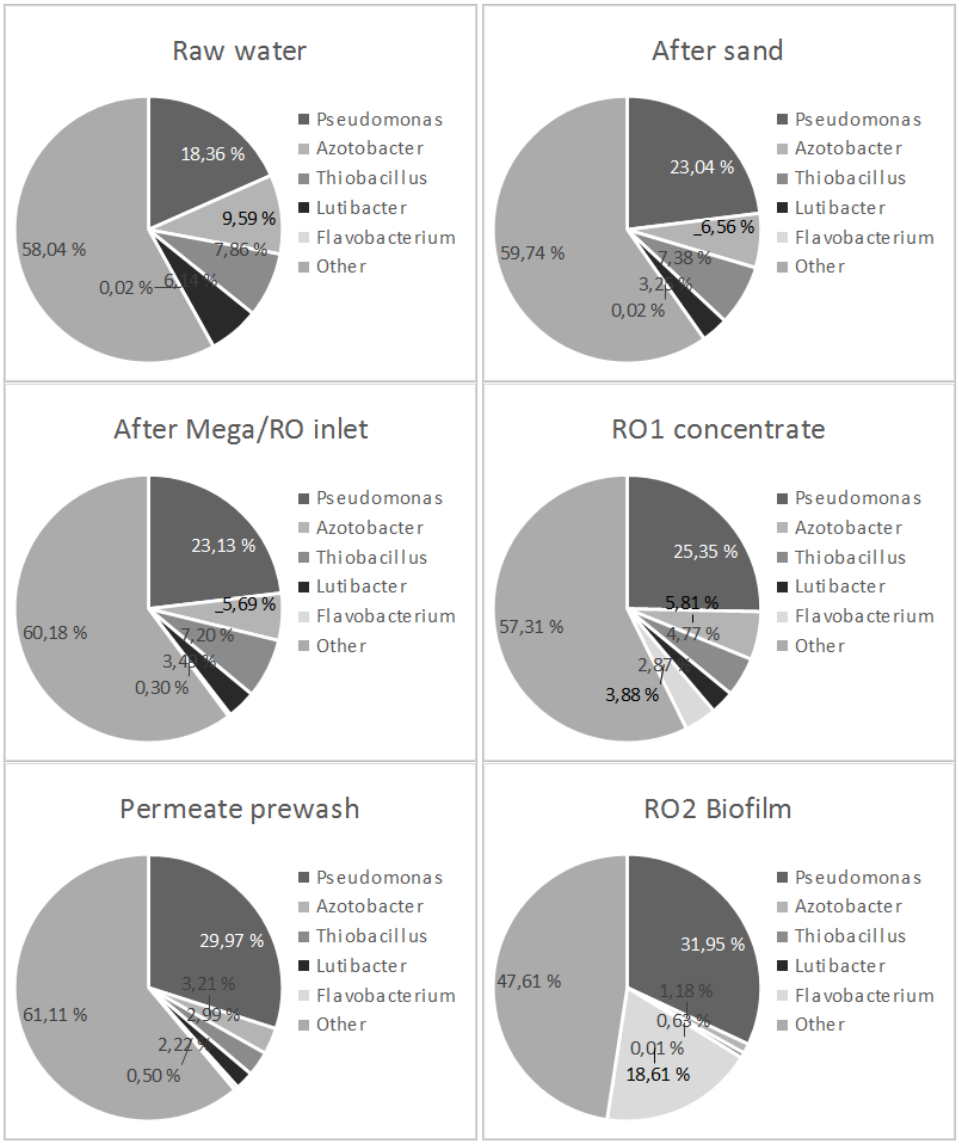

***Figure 3**. Major bacterial species (OTUs) in RO waters and biofilm samples.*

the washing water which is seen as extremely high bacterial levels with *Pseudomonas* as a dominant bacterial genus.

The bacterial community analysis by NGS revealed the dominance of *Pseudomonas* and *Flavobacterium* in a RO biofilm collected two months earlier than the water samples. As *Pseudomonas* spp. was detected as a dominant genus from both of the biofilms of November and the washing water in January, it might serve as a good indicator for biofouling in this system.

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