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**Characterisation of (bio)fouling on used reverse osmosis membranes**

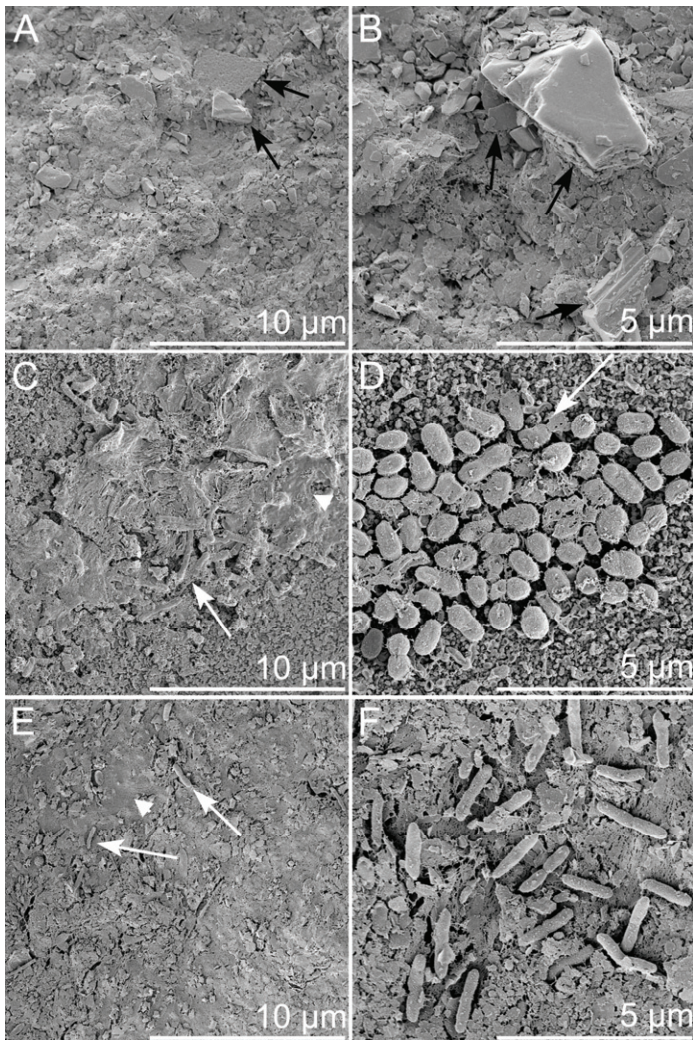
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**Introduction:** Biofouling is one of the most critical problems in sea/brackish water desalination plants. In desalination processes biofouling has been detected to cause flux reduction, increased operational pressure, pressure drop, energy efficiency reduction and membrane damage. Until now the research has mainly been focusing on characterization of biofouling on brackish water, seawater and wastewater treatment applications. These studies are mainly limited to one or two plants and there is a lack of a general study on characterization of microbes on different elements from different plants. Biofilm formation depends on interaction between the bacterial cells, the attachment surface and the surrounding medium. In addition, bacterial cell surface properties (e.g. cell surface hydrophobicity, extracellular polymers and appendages) influence on their potential to adhere on surfaces.

In order to develop novel antifouling agents and biocides information about microbes causing biofouling and their interactions with the surfaces is vital. Aim of this study was to characterize biofouling occurring on SWRO and BWRO membranes both on membrane and spacer samples by molecular biology methods (Polymerase Chain Reaction- Denatured Gradient Gel Electrophoresis, PCR-DGGE) and with microscopic techniques (FESEM).

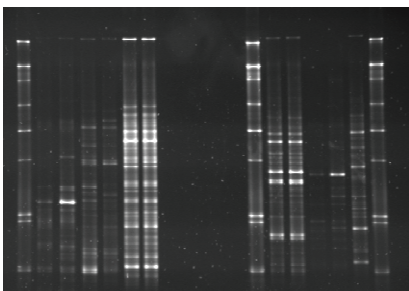
**Methods:** Five BWRO and three SWRO membranes originating from different parts of the world with field emission scanning electron microscopy (FESEM) (Raulio et al. 2006) were examined. DNA was extracted from the samples with FastDNA Spin Kit for Soil (MP Medicals). V6-V8 region of the 16S rRNA gene was amplified using the primer pair F-968-GC and R-1401 (Nübel et al. 1996) as described by Laitila et al. (2007). DGGE analysis was performed with D-Code Universal Mutation Detection System (BioRad Laboratories GmbH). The gels were stained with SYBR<sup>TM</sup> Green I (Sigma) and documented using the Gel Doc<sup>TM</sup> 2000 system (BioRad). Selected bands from DGGE analyses were identified further with partial 16SRNA gene sequencing. Excision, purification and sequencing of DNA fragments from DGGE gels were performed as described by Laitila et al. (2007). Our aim was to identify biological fouling causing bacteria and characterise scaling and biofouling layers on the membrane surfaces. Both membrane and spacer samples were examined.

**Results and discussion:** Microscopical examination of the fouled membranes revealed heavy chemical and biological fouling (Figure 1). Microbial slime was observed in several locations of the examined sample surfaces and they seem to significantly contribute to the structure of biofouling layers. Membranes were more heavily biofouled compared to spacers. The FastDNA extraction efficiently released DNA from the spacer and membrane samples. Typical PCR-DGGE analysis profile of the examined samples is presented in Figure 2. The 16S rRNA gene sequencing and preliminary identification results from the samples indicate the dominance of *Gammaproteobacteria* belonging to the genus *Alkanibacter* in the SW membrane samples. Also other *Proteobacteria* were detected. For BW membrane samples the preliminary results indicate presence of marine *Gammaproteobacteria*, e.g. *Xanthomonas* and *Pseudomonas* on BW membranes. Result obtained with FESEM and PCR-DGGE describing characteristics of fouling layer on various membranes were comparable in most cases.



**Figure 1.** Examples of SEM micrographs of fouled membranes. Panels A and B shows membrane fouled by inorganic scaling with plate like morphology and large crystal like structures (black arrows). Panels C and D show a membrane that was only partly covered with fouling layer and many areas were totally free of any fouling layer. Many microbial aggregates with microcolony appearance were observed (white arrows) adhered on otherwise clean membrane surface. Some of the microbes were fully covered by extracellular substance (white arrow heads). In Panels C and D micrographs of membrane with thick fouling layer consisting mostly of microbial cells (white arrows) and substances produced (white arrow head) by them are shown.

M 1a, 1b 2a, 2b 3a, 3b 4a, 4b 5a 5b M 6a 6b 7a 7b 20a M



**Figure 2.** Typical PCR-DGGE pattern of fouled membranes. Samples 1-5 brackish water membranes and samples 6, 7 and 20 sea water membranes. M= marker.

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