Accepted Manuscript

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PII: S0043-1354(16)30098-7

DOI: [10.1016/j.watres.2016.02.039](https://doi.org/10.1016/j.watres.2016.02.039)

Reference: WR 11855

To appear in: *Water Research*

Received Date: 19 September 2015

Revised Date: 10 February 2016

Accepted Date: 15 February 2016

Please cite this article as: Belila, A., El-Chakhtoura, J., Otaibi, N., Muyzer, G., Gonzalez-Gil, G., Saikaly, P.E., van Loosdrecht, M.C.M., Vrouwenvelder, J.S., Bacterial community structure and variation in a full-scale seawater desalination plant for drinking water production, *Water Research* (2016), doi: 10.1016/j.watres.2016.02.039.

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seawater desalination process
bacterial community analysis by 454 pyrosequencing

Bacterial community structure and variation in a full-scale seawater desalination plant for drinking water production

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Abstract

Microbial processes inevitably play a role in membrane-based desalination plants, mainly recognized as membrane biofouling. We assessed the bacterial community structure and diversity during different treatment steps in a full-scale seawater desalination plant producing 40,000 m³/d of drinking water. Water samples were taken over the full treatment train consisting of chlorination, spruce media and cartridge filters, de-chlorination, first and second pass reverse osmosis (RO) membranes and final chlorine dosage for drinking water distribution. The water samples were analyzed for water quality parameters (total bacterial cell number, total organic carbon, conductivity, pH, etc.) and microbial community composition by 16S rRNA gene pyrosequencing. The planktonic microbial community was dominated by *Proteobacteria* (48.6%) followed by *Bacteroidetes* (15%), *Firmicutes* (9.3%) and *Cyanobacteria* (4.9%). During the pretreatment step, the spruce media filter did not impact the bacterial community composition dominated by *Proteobacteria*. In contrast, the RO and final chlorination treatment steps reduced the *Proteobacterial* relative abundance in the produced water where *Firmicutes* constituted the most dominant bacterial group. Shannon and Chao1 diversity indices showed that bacterial species richness and diversity decreased during the seawater desalination process. The two-stage RO filtration strongly reduced the water conductivity (>99%), TOC concentration (98.5%) and total bacterial cell number (>99%), albeit some bacterial DNA was found in the water after RO filtration. About 0.25% of the total bacterial operational taxonomic units (OTUs) were present in all stages of the desalination plant: the seawater, the RO permeates and the chlorinated drinking water, suggesting that these bacterial strains can survive in different environments such as high/low salt concentration and with/without residual disinfectant. These bacterial strains were not caused by contamination during water sample filtration or from DNA extraction protocols. Control measurements for sample contamination are important for clean water studies.

Key words

16S rRNA gene pyrosequencing; bacterial population; sample contamination control; seawater desalination; reverse osmosis; membrane based drinking water production

Abbreviations: bTEFAP, bacterial tag-encoded FLX amplicon pyrosequencing; BWRO, brackish water reverse osmosis; DPD, N, N-Diethyl P-Phenylenediamine; LSI, Langelier saturation index; NTU, nephelometric turbidity units; OTU, operational taxonomic units; PCoA, principal coordinate analysis; RO, reverse osmosis; SBS, sodium bisulphite; SWRO, seawater reverse osmosis; SD, standard deviation; TOC, total organic carbon; UwUnF, unweighted UniFrac; WUnF, weighted UniFrac.

1. Introduction

In coastal areas around the world seawater desalination is becoming increasingly important as a source of drinking water. The total desalination capacity worldwide using reverse osmosis (RO) membrane technology is the largest compared to other desalination processes including multistage flash distillation, multiple-effect distillation, vapor compression, and electrodialysis. The global RO capacity is continuously increasing with time (Ghaffour et al., 2013). In addition, the strong reduction with time in the costs of membrane-based desalination has enabled many countries to implement desalination for potable water supply.

The variation of the microbiological quality of the drinking water during production and distribution processes has long been a crucial issue since many problems in those systems – including desalination systems – are microbial in nature, including biofilm growth, pathogen persistence and biofouling (Berry et al., 2006). Determining the microbial ecology of drinking water production systems is imperative, as recent investigations have found that the resistance of pathogens to chlorination is affected by the bacterial community diversity and interspecies relationships (Kormas et al., 2010.). Several studies have discussed the microbial ecology and dynamics of drinking water supply systems (El-Chakhtoura et al., 2015), but the majority (even in full-scale desalination plants) focused on the exploration of the biofilm microbial communities on RO membranes and cartridge filters (CF) and their role in biofouling (Zhang et al., 2011; Chiellini et al., 2012) with the aim to improve treatment efficiency and plant performance (Bereschenko et al., 2008). Few studies have characterized planktonic communities in full-scale water supply systems supplemented with different source waters, e.g. surface and groundwater (Eichler et al., 2006; Pinto et al., 2012) and discussed how the treatment train shapes the planktonic microbiome and influences the final bacterial communities.

In this study, we applied 16S rRNA gene pyrosequencing to: (i) identify and characterize the planktonic bacterial communities associated with different compartments of a full-scale desalination plant for drinking water production and (ii) compare these communities across different locations throughout the water treatment process. To the authors' knowledge, this is the first study that investigates the microbial ecology of a seawater desalination plant for drinking water production.

2. Materials and methods

2.1. KAUST desalination plant

The desalination plant is located at King Abdullah University of Science and Technology (KAUST) in the West of the Kingdom of Saudi Arabia. The facility was designed to provide all potable water needs for the campus as well as the residential areas. The plant has a drinking water production capacity of 40,000 m³ per day. The source is from the Red Sea. The desalination plant consists of three parts: pre-treatment, reverse osmosis (RO) system and post-treatment.

Pre-treatment involves the sequence of (i) open seawater intake, (ii) fine screen filtration, (iii) water chlorination at the intake point, and filtration through (iv) spruce multi-media filters (SMF to remove particulate and colloidal matter). The water is chlorinated once a week for a period of 1 h to prevent microbial growth on the inside of the water intake pipe and the SMF. Thereafter, a portion of the water flow is directed to fill the SMF backwash water tank (an SMF is backwashed every 12 h for a period of $\approx$ 30 minutes) while the remaining water flow is directed towards the RO treatment trains (Figure 1).

The RO system involves the sequence of (i) anti-scalant dosage (1.5 mg/L, to avoid the RO system from inorganic deposition, also named scaling), (ii) cartridge filtration (10 μ m pore size, to protect the RO from particulate fouling) and (iii) sodium bisulfite (SBS) dosage (to neutralize chlorine residual that may compromise the RO membrane integrity) and then (iv) the RO installation consisting of a seawater (SWRO) and brackish water RO (BWRO) pass.

The first pass SWRO produces the design fresh water quality goals except for Boron, while the second pass BWRO treatment is needed to lower the Boron concentration. The SWRO produced water passes a break tank (8000 m³) and is subsequently fed with caustic soda to increase the pH in order to increase Boron rejection in the second pass treatment (BWRO). Addition of caustic soda will shift the equilibrium from B(OH)₃ to B(OH)₄⁻. Because of the larger sized B(OH)₄⁻ ions, higher Boron rejection is achieved by the BWRO membranes. The SWRO pass has four trains, each with 140 pressure vessels of 7 membrane modules, making a total of 3920 membrane modules. 8 inch diameter Toray TMC820C-400 membrane modules with 34 mil thick feed spacer, a membrane surface area of

37 m² and salt rejection properties of 99.75% were used. The water recovery of the SWRO pass is 40%. The inlet pressure is about 60.6 bar and the reject pressure about 59.4 bar. Conductivity of the SWRO feed is about 56.47 mS/cm and that of the SWRO permeate is about 700 µS/cm (note the unit). The membrane differential pressure is about 1.2 bar across one pressure vessel.

The second pass BWRO has 4 trains, each with 34 pressure vessels of 7 membrane modules, making a total of 952 membrane modules. 8 inch diameter Toray TM720-440 membrane modules with 28 mil feed spacer thickness, a membrane surface area of 40 m² and salt rejection properties of 99.7% were used. The water recovery of the BWRO pass is 90%. The inlet pressure is about 10.5 bar and the reject pressure about 7.5 bar. Conductivity of the BWRO permeate is about 30 µS/cm. The membrane differential pressure is about 3 bar across one pressure vessel. Both the SWRO and BWRO membrane types are cross linked fully aromatic polyamide composite in a spiral wound configuration.

Post-treatment: About 60% of the BWRO permeate is mixed with 40% of the SWRO permeate to meet the World Health Organization health-based guideline for Boron. Finally, the produced water (mix of BWRO and SWRO permeates) is chlorinated to maintain 0.5-1.0 mg/L of residual chlorine. Post-chlorination is applied to avoid microbial growth during distribution. In addition, the following chemicals are dosed (i) hydrated lime (43 mg/L) for water conditioning to prevent pipe corrosion and (ii) CO₂ (20 mg/L) to adjust the pH to near a neutral value with positive Langelier saturation index (LSI). Subsequently, the produced drinking water is pumped into storage reservoirs (two vessels, each 10,500 m³) feeding the drinking water distribution network. Water quality analysis data is shown in Table 1 (selected) and Table S1 (raw seawater, SWRO and BWRO permeate and product) in the supplementary material. The overall water recovery of the plant is 38%. The water production (O&M) costs are USD 0.40 /m³ (IDA, 2015).

Hydraulic retention time (hydraulic residence time) of water in the break tank and the drinking water reservoirs: The break tank (8000 m³) provides (i) make up water for the chillers (250 m³/h), (ii) blending water for the BWRO permeate (680 m³/h), and (iii) BWRO feed water (1100 m³/h), indicating a 4 h retention time in the break tank. Daily, about 40,000 m³ (1667 m³/h) drinking water passes the storage tanks (21,000 m³), indicating a 12.6 h retention time in the drinking water storage tanks.

2.2 Water sampling and sample IDs

Six water samples were collected on the same day (6 August 2012) from the desalination plant using 4 L sterile polyvinyl chloride plastic bottles at the following locations: inlet seawater (TR1), spruce media filter permeate (TR2), seawater reverse osmosis feed (TR3), seawater reverse osmosis permeate (TR4), brackish water reverse osmosis permeate (TR5) and final chlorinated drinking water (TR6) (Figure 1). The labels TR1 to TR6 are used to indicate the water sampling locations. The water samples were kept on ice until analysis. Within 24 hours after sampling, the water samples were analyzed for water quality parameters and filtered for microbial community analysis.

2.3 Water quality analysis

The water quality was analyzed through the measurement of pH (Cyberscan pH6000, Eutech, USA), conductivity (CON 510, Oakton, USA), Total Organic Carbon (TOC; TOC-VCPH Analyzer, Shimadzu, Japan) and residual chlorine concentration which was measured by the DPD method using a pocket colorimeter TM II (HACH). Flow cytometry analysis of the total bacterial cell concentrations was done by SYBR Green I cell staining followed by cell count in a defined sample volume using an Accuri C6 Flow Cytometer (BD Biosciences) as previously described (Berney et al., 2008; Hammes et al., 2008; Prest et al., 2013, 2014).

2.4 DNA extraction, quantification and control measurements

Water samples of 3 L from each of the locations TR1-TR6 were filtered through polycarbonate membranes (pore size 0.2 μ m, membrane diameter 45 mm, Millipore) which were rolled and placed into Cryo-vials (Nalgene, Nunc, Rochester, NY, USA), and frozen at -80 °C until DNA extraction. DNA was extracted from the membranes using a FastDNA spin kit for soil (FastDNA, MP Biomedical, Illkirch, France). The extracted DNA was concentrated in 50 μ L DNAase-free water (extracted from 3 L water). The DNA concentration was quantified using Nanodrop 2000c (www.nanodrop.com, Wilmington, DE 19810, USA) and expressed as ng/ μ l in a total elution volume of 50 μ L.

Low extracted DNA concentrations were expected in the water samples after reverse osmosis filtration (TR4-TR6). Therefore, controls were applied to quantify possible contamination of the samples with DNA from the materials used and the environment (e.g. sample handling, extraction kit, water filtration unit, membrane and air contact). As controls, (i) virgin membranes without water and (ii) virgin membranes filtering 0.5 L of DNA-free water were applied. For the control samples, the same DNA extraction and quantification method was applied as for the water samples taken at the locations TR1-TR6.

2.5. PCR and 16S RNA gene pyrosequencing analysis

The V4-V5 hypervariable region of the bacterial 16S rRNA genes was amplified using the PCR primers 515F and 907R (Zhou et al., 2011). Both primers covered >98% of the 16S gene sequences in the Ribosomal Database Project (Cole *et al.*, 2009). A single-step 30 cycles PCR using HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA) was used with the following conditions: 94°C for 3 minutes, followed by 30 cycles of 94°C for 30 seconds; 53°C for 40 seconds and 72°C for 1 minute; after which a final elongation step at 72°C for 5 minutes was performed. Following the PCR, all amplified products from the different samples were mixed in equal concentrations and purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, USA). Samples were sequenced utilizing Roche 454 FLX titanium instruments (Roche, Indianapolis, IN) and reagents and following the manufacturer's guidelines.

2.6. Data processing

The sequences were processed using a proprietary analysis pipeline (www.mrdnalab.com, MR DNA, Shallowater, TX, USA). Sequences were depleted of barcodes and primers. Subsequently, sequences shorter than 200 bp, sequences with ambiguous base calls, and sequences with homopolymer runs exceeding 6 bp were removed. Sequences were then denoised and checked for chimera sequences. Operational taxonomic units (OTUs) were defined after removal of singleton sequences, clustering at 3% divergence (97% similarity) (Eren et al., 2011; Swanson et al., 2011). OTUs were then

taxonomically classified using BLASTn against a curated GreenGenes database (DeSantis et al., 2006).

2.7. Alpha and beta diversity measures

Rarified OTU tables were used to generate alpha and beta diversity metrics. Shannon diversity index (H) and species richness estimator of Chao1 were generated for each sample using QIIME as a measure of alpha diversity. Spatial variation of bacterial community was analyzed with principal coordinate analysis (PCoA) in QIIME using unweighted UniFrac distance matrix (Lozupone et al., 2010) as a measure of beta diversity.

Shared and sample-specific OTUs were calculated using Venny online software (Oliveros, 2007) (<http://bioinfogp.cnb.csic.es/tools/venny/index.html>) and Venn diagrams were constructed using Microsoft Office PowerPoint.

3. Results

3.1. Water parameters

The mean water temperature in the seawater desalination process was 24.4 ± 0.72 °C and ranged between 25.6°C and 23.5°C (Table 1). The TOC concentration ranged between 1.68 mg/L in the inlet water (TR1) and 20 µg/L in the final chlorinated water (TR6): 98.5 % of the total organic carbon was removed during seawater desalination. The increase in pH to 10.1 in TR4 is due to the addition of NaOH and anti-scalant necessary to enhance the removal of Boron. The water conductivity was reduced by 98.5% in the first RO pass while the second RO pass reduced conductivity by 86.2% resulting in an overall conductivity reduction of 99.8%. No residual chlorine was detected during the desalination process. After chlorination, we detected 0.50 mg/L free residual chlorine in the produced drinking water. All measured parameters of the final product water were in accordance with World Health Organization physicochemical quality standards for drinking water (WHO, 2004).

Based on flow cytometry results, 40% of the total bacteria in the inlet water ($5.47 \times 10^4 \pm 860$ cells/mL) were retained by the spruce media filter during the pretreatment step while 13% of the rest of the bacterial cells were removed by the cartridge filter. The total number of bacteria in the water decreased by $\geq 99.3\%$ after the first reverse osmosis filtration step and remained below the detection limit (≤ 200 cells/mL) of the flow cytometer in TR4, TR5 and TR6 (Table 1).

3.2. Bacterial diversity

A total of 252,648 sequence reads were obtained from all sampling sites with an average length of 379 bp. Of the total reads, 49,213 reads (19.4%) were removed after trimming and chimera check. For the downstream analyses, only OTUs with 97% similarity cut-off were used. The species richness estimator Chao1 was up to 2176 in the spruce media filter permeate water (TR2) (Table 2). The Chao1 index decreased during the pre-treatment step from 2083 (TR1) to 1641 (TR3) and then dropped to 445 after the first RO pass (SWRO pass). After the second RO pass (BWRO pass), the Chao 1 richness increased to 574 and 614 in TR5 and TR6, respectively. The Shannon diversity index showed the same trend during the pre-treatment step and decreased progressively from 8.59 (TR1) to

7.98 and 7.75 in TR2 and TR3, respectively. The bacterial species diversity increased slightly after the first RO pass to 7.97, then dropped to 7.24 after the second RO pass and to 6.08 after chlorination of the drinking water (Table 2).

Rarefaction curves did not reach a plateau for samples TR1, TR2 and TR3 as OTUs continued to emerge for some samples even after 8,000 reads, suggesting that there was additional diversity in those samples that was probably not captured by pyrosequencing. In contrast, rarefaction curves for samples TR4, TR5 and TR6 reached a plateau after 6,000 reads, reflecting a more limited bacterial diversity (Figure S1 in supplementary material).

3.3. Microbial community structure

Principal coordinate analysis (PCoA) based on unweighted UniFrac showed three separate clusters consisting of pre-treatment water samples (TR1, TR2 and TR3), RO permeate samples (TR4 and TR5) and the final chlorinated drinking water (TR6) (Figure 2). PCoA revealed a clear separation in the microbial community structure between pre- (TR1, TR2 and TR3) and post-RO treatment (TR4 and TR5) samples along principal component 1 (PC1) while the effect of chlorination is seen along principal component 2 (PC2) where there is a separation between TR4/TR5 and TR6 (Figure 2).

3.4. Taxonomic diversity

Diverse bacterial phyla were detected at all sampling sites (Figure 3). The bacterial community was dominated by *Proteobacteria* (48.6%) followed by *Bacteroidetes* (15.0%), *Firmicutes* (9.3%), *Cyanobacteria* (4.9%), *Planctomycetes* (3.0%) and *Actinobacteria* (1.9%).

Six Proteobacterial classes were identified within all processed samples with *Alphaproteobacteria* (29.5%) being the most dominant followed by *Gammaproteobacteria* (14.7%), *Deltaproteobacteria* (2.7%), and *Betaproteobacteria* (2.3%). When moving from the seawater intake side of the desalination plant to the produced drinking water, the relative abundance of *Alphaproteobacteria* increased in the first three sampling locations (TR1, TR2 and TR3) and reached 74.1% then decreased to 31.4% after the first RO pass and finally dropped to 25.0% in the produced drinking water. While present in all sampling sites, the relative abundance of *Betaproteobacteria* reached the highest value

in the produced drinking water at 45.3%. The relative abundance of *Deltaproteobacteria* varied between 0.94% (TR5) and 9.2% (TR4). For a more detailed characterization of the *Proteobacteria* community composition we analyzed the taxonomic structure down to the order level as shown in Figure 4. The *Alphaproteobacteria* were dominated by *Sphingomonadales* (1.5% - 41%), *Rhodobacterales* (SAR86 clade) (23.5% - 57%), *Rhizobiales* (0.8% - 48%), and *Rickettsiales* (SAR11 clade) (5.0% - 70%) (Figure 4a). The *Betaproteobacteria* exhibited the highest community structure stability during the desalination process and was dominated by the order of *Burkholderiales* (Figure 4b). The *Gammaproteobacteria* were the most diverse Proteobacterial group and were dominated by the order *Alteromonas* (3.5%-27%), *Pseudomonadales* (24.5% - 65%), *Oceanospirillales* (7.5% - 39%) and the phototrophic anoxygenic purple bacteria of the order *Chromatiales* (1.4% - 16.5%) (Figure 4c). The *Deltaproteobacteria* were dominated by *Desulfobacterales* (9.0% - 29%), *Myxococcales* (11.0 - 47%) and *Desulfomonadales* (8.8% - 52%) (Figure 4d).

The most dominant non-Proteobacterial classes for all sampling locations combined in descending order of their relative abundance were *Synechococcophycideae* (15.3%), *Flavobacteria* (15.3%), *Clostridia* (11.1%), *Bacteroidia* (7.8%), *Sphingobacteria* (6.2%), *Cytophagia* (6.2%), *Planctomycetacia* (5.0%), *Bacilli* (2.8%), *Unclassified Actinobacteria* (1.8%) and *Opitutae* (1.7%). The *Synechococcophycideae*, a class in the phylum *Cyanobacteria* was one of the most abundant non-Proteobacterial classes especially during the spruce and cartridge filtration where the relative abundance reached 46.4% in the spruce media filter permeate (TR2) and then declined to less than 1% in the water after RO (TR5 and in TR6). The *Flavobacteria* were one of the most abundant non-Proteobacterial class detected at all sampling sites before the RO treatment. Their relative abundance was 30%, 25% and 19% at TR1, TR2 and TR3, respectively (Figure 5). Despite their presence within all water samples, the relative abundance of *Bacilli* increased remarkably from 0.4% in TR3 to 9.6% and 6.0% in the RO permeates (TR4 and TR5, respectively). In TR6 the *Bacilli* class represented 5.9% of the total non-Proteobacterial community. The *Planctomycetacia* were present only within the three first sampling sites (TR1, TR2 and TR3), while the *Sphingobacteria* were detected at all sampling locations (Figure 5). The relative abundance of the *Bacteroidia* increased downstream the RO treatment process from 17.6% in TR4 to 26.8% in TR5 then decreased to 20.0% in TR6.

3.5. Core and unique OTUs

The presence of core OTUs at all stages of the desalination plant was determined and analyzed. Commonly occurring bacteria that appear in all assemblages associated with a particular habitat are critical for the function of the community. Thus, identifying a core community may help in predicting community responses to a perturbation (e.g. filtration, chlorination) (Shade and Handelsman, 2012). Venn diagrams were used to assess the core OTUs during seawater processing (Oliveros, 2007). For this, OTUs (97% cutoff) were classified in three groups: Core OTUs, detected in all sampling locations; Variable OTUs, not detected in all locations, but found in more than one location, and Unique OTUs, defined as OTUs detected in one sampling location. For the 11728 OTUs detected at all sampling locations 54.3%, 45.5% and 0.25% were classified as Unique, Variable and Core OTUs, respectively. Only 3 bacterial species were detected in all six sampling locations over the full treatment train and were identified as *Rhodobacteraceae* spp., *Candidatus Pelagibacter ubique*, and the cyanobacterial species *Prochlorococcus* spp. The Venn diagram showed that 811 OTUs were shared between the bacterial communities collected from the three sampling locations prior to RO filtration (TR1, TR2 and TR3) (Figure 6a), 361 OTUs were shared between bacterial communities from TR5 (BWRO permeate) and TR6 (the final chlorinated drinking water) (Figure 6c) while 66 OTUs were shared between TR3, TR4 and TR5 (Figure 6b). About 0.25% of the total bacterial OTUs was present in all stages of the desalination plant: the seawater, the RO permeates and the chlorinated drinking water, suggesting that some bacterial strains can survive in strongly different environments such as high/low salt concentrations and without/with residual disinfectant. Most likely these bacteria did not pass the RO membranes (no leakage), suggesting that the bacteria were present at the start-up of the installation (e.g. by placement of the RO membrane elements in the pressure vessels) and/or were introduced later as contamination from the environment (e.g. by maintenance and inspection of the RO installation including the reservoirs).

3.6. Sample contamination

Low DNA concentrations and low OTU numbers were expected in the water after reverse osmosis filtration. The DNA concentration is presented in ng/μl DNA in a final volume of 50 μL, extracted

from 3 L of water. The DNA concentration decreased by 30% after the spruce media filter (from 200 ng/ μ L to 153 ng/ μ L) and by 78% (from 133 ng/ μ L to 30 ng/ μ L) after the first RO step. Remarkably, the DNA concentration remained stable in both RO permeates (30.0 ng/ μ L in TR4 and 26.6 ng/ μ L in TR5) and in the final produced drinking water (27.1 ng/ μ L in TR6) despite the decrease in the total bacterial cell number accounted by flow cytometry (Table 1). Therefore, to quantify possible DNA contamination by water sample processing, a series of control measurements were done with the same materials for bacteria collection (filter and filter unit) without and with filtration of DNA-free water. In all control samples, (i) DNA concentrations (ranging from 9 to 10 ng/ μ L) were much lower than in the water samples, and (ii) an average OTU number of 72 ± 5 was detected. Although the fraction of shared OTUs between the 8 control samples was 46% (30 out of 72), these shared OTUs corresponded to a high percentage (~80%) of the total sequence reads, implying the detected bacteria were similar in the different control samples. The OTU numbers in the control samples were much lower than in the water samples (Table 2). The contamination of the water sample OTUs with OTUs from the sample processing is in the range of 0.9% to 6.0% of the total OTUs found, indicating that nearly all of the OTUs (94%-99%) and the DNA originate from the water samples itself. The bacterial species identified in all control samples differed from the bacterial species found in all water samples over the treatment train, indicating that the three common bacterial strains (0.25% of OTUs) in the treatment plant were not caused by contamination from water sample filtration and DNA extraction protocols (Table S2 in supplementary material). These results suggest that the origin of the three bacterial species is the water. Bacterial growth or intrusion after reverse osmosis may occur as suggested by the detection of some OTUs affiliated with non-marine microbes and detected only in the final stage (TR6) of the treatment train (Table S3 in supplementary material).

4. Discussion

4.1. Microbial community diversity and structure

16S rRNA genes pyrosequencing of water samples at different stages of the desalination plant revealed taxonomically diverse bacterial communities. The diversity of planktonic bacteria declined after the spruce media filter, after each RO unit, and after chlorination (Table 2), indicating that water treatment caused an alteration in the diversity of the planktonic bacterial community (Kormas et al., 2010; Pinto et al., 2012). This is supported by the fact that the water chemical and physical qualities changed after each treatment step along the desalination process (Table 1). Principal coordinate analysis revealed spatial variation in the bacterial community structure (Figure 3) suggesting the role of different treatment processes and salinity changes in shaping the microbial communities during drinking water production.

4.2. Interpretation of bacterial community composition

The pyrosequencing analyses revealed that the desalination plant hosted a high bacterial diversity and that distinct communities were detected at each step of the desalination process despite the effectiveness of the RO process in removing bacterial cells from the water. This bacterial community was predominated by *Proteobacteria*, *Bacteroidetes*, *Firmicutes* and *Cyanobacteria*. Similar bacterial community assemblages have been previously described in marine ecosystems (Manes et al., 2011; Elifantz et al., 2013). Moreover, *Alphaproteobacteria*, *Gammaproteobacteria* and *Bacteroidetes* have been shown to be the most dominant bacterial assemblage in coastal marine ecosystems (Dang et al., 2008). *Cyanobacteria* strains have been isolated from drinking water reservoirs and tap water samples (Codony et al., 2003; Izaguirre et al., 2007; Revetta et al., 2011), suggesting that there might be some cyanobacterial groups that not only can withstand disinfection treatment but might have physiological capabilities that enhance their survival in the absence of light.

4.2.1. Proteobacterial community in desalination plant

Members of the *Alphaproteobacteria* were dominant in the water prior to RO filtration (TR1, TR2 and TR3) and in the produced drinking water (TR6) while *Gammaproteobacteria* were dominant in the seawater reverse osmosis permeate (TR4). After chlorination (TR6) the bacterial community was dominated by the *Betaproteobacteria* (45.3%) which contrasts with previous findings showing *Alphaproteobacteria* to be the dominant group in treated water samples due to their capacity to survive in low nutrient and chlorinated environments, while *Betaproteobacteria* were observed more frequently in water with low disinfectant residuals (Chao et al., 2013; El-Chakhtoura et al., 2015). However, a previous study showed that 0.4 mg L⁻¹ of residual chlorine was a sensitive oxidant level to the *Alphaproteobacteria* population and could favor *Beta*- and *Gammaproteobacteria* which normally are favored by higher free residual chlorine concentrations (Mathieu et al., 2009). The treatment train was distinctly effective in removing *Proteobacteria* (Figure 3, from ca 70% before RO to ca 10% in the final water (TR6) with cell numbers below flow cytometry detection limits (Table 1) and in tandem the *Gammaproteobacteria* class was considerably reduced throughout all treatment steps (3.9% in TR6).

Within the *Alphaproteobacteria*, the increase in *Rhizobiales* relative abundance within the SWRO and BWRO permeate water (TR4 and TR5) may indicate bacterial growth and/or the detachment of bacteria from biofilm present (i) at the permeate side of the RO membranes, (ii) in the pipes and (iii) in the reservoir. In fact, *Rhizobiales* represents one of the largest bacterial fractions in bench-scale RO membranes and full-scale biofilms from water purification systems and are known to produce extracellular polymeric substances (EPS) providing a protective environment for microbial cells to grow and persist in biofilms (Pang and Liu, 2007). *Rhodobacterales* with the SAR11 clade (Rickettsiales) were the most dominant alphaproteobacterial group at all sampling sites (except in TR6). The former group is a rapid primary surface-colonizer in coastal waters (Zhang et al., 2006; Dang et al., 2008) participating in bio-corrosion and biofouling phenomenon (Beech et al., 2005). Their ubiquitous presence in all sampling sites may be linked to the presence of biofilm in all compartments of the desalination plant. In contrast, the SAR11 clade members are considered to be planktonic (Giovannoni and Stingl, 2005). All *Betaproteobacteria* were identified as members belonging to the order *Burkholderiales* and in particular to the *Burkholderiaceae* and

Comamonadaceae families. This bacterial group was described previously as a major component of the RO membrane biofilm (Xia et al., 2010; Al Ashhab et al., 2014). Within the *Deltaproteobacteria*, phylotypes belonging to the *Desulfarculales*, *Desulfobacterales*, and *Desulfomonadales* orders were detected. These bacterial groups may act as sulfate reducers or metal oxidizers that could affect water distribution pipe integrity (Seth and Edyvean, 2006).

4.2.2. Effect of filtration process on bacterial community structure

Distinct bacterial communities were detected in the water at each step of the water treatment plant. It is likely that particular operations employed during the drinking water production process affected the bacterial composition in distinctive ways (Pinto et al., 2012). Based on the Alpha diversity index (Table 2), the bacterial diversity remained unchanged after the seawater reverse osmosis (SWRO) pass, but decreased after the following brackish water reverse osmosis (BWRO) pass and after the disinfection step of the water before distribution. Thus, the impact of the BWRO and chlorination was more pronounced on the bacterial diversity than the impact of the SMF process and the SWRO. On the other hand, while the SMF and chlorination showed no effect on the species richness (Chao1 index), it was clear that both the SWRO and BWRO reduced the bacterial species richness of the planktonic bacterial community. Both types of treatments (RO and chlorination) had distinct effects on the bacterial species richness during the drinking water production process.

4.2.2.1. Effect of spruce media filter

The SMF is an improved system for lowering suspended solids and microorganisms from seawater. It is a multi-media deep bed filter consisting of 4 layers of natural, long lasting, inert media with an increasing density, decreasing particle size and specific shape factor able to achieve submicron particle filtration ranging between 0.2 and 50 μm . The main objectives of the pretreatment filtration step are the mechanical retention of the suspended solids and adsorption of negatively charged microorganisms. After the SMF treatment step, 34%, 33% and 45% of the total organic carbon, water turbidity and total cell concentration were removed by the SMF, respectively. Crossing the SMF, *Proteobacteria* remained the dominant phylum slightly increasing in the water (67% (TR1) to 73%

(TR2)). The *Alphaproteobacteria* dominated the Proteobacterial community with a relative abundance of 68.9%. Also, the relative abundance of *Planctomycetes* and *Bacteroidetes* decreased in the water. In contrast, *Cyanobacteria* abundance increased after the SMF filtration step (from 3.0% to 14.3%). At the class level, the relative abundance of *Synechococcophycidae*, *Cytophagia* and *Flavobacteria* increased after the SMF step, while *Clostridia* and *Planctomycetacia* abundance decreased.

4.2.2.2. Community structure after first RO pass

At this SWRO step, 29% of the TOC was removed. $\geq 99\%$ of the total bacterial cell concentration was removed after the 1st RO pass. The most relevant changes within the bacterial community composition observed were the decrease by 50% of the relative abundance of the *Proteobacteria*, and the increase in the relative abundance of *Firmicutes*, *Bacteroidetes* and *Planctomycetes*, possibly by growth and/or detachment. Indeed, the *Bacteroidetes* group likely plays a role in the degradation of polymeric organic matter and is found in marine pelagic zones and often associated with surfaces (Frette et al. 2004). *Bacteroidetes* are known to constitute one of the components of the fouling layers on SWRO-membranes (Rapenne et al., 2009) since they are well adapted to grow on surfaces and could play a role in degrading biopolymers. In contrast, in our study, they constituted the second major group in the bulk water. *Cyanobacteria* have been identified among potentially active bacteria in chlorinated drinking water suggesting that they survive treatment disinfection and harsh conditions in distribution systems and have been shown to break through filtration treatment (Dugan and Williams, 2006). Crossing the first reverse osmosis pass, *Gammaproteobacteria* dominated the *Proteobacteria* community. In addition, the abundance of the *Beta*- and *Deltaproteobacteria* increased in the produced water. An increase in the abundance of *Bacteroidia* (from 0.5% to 17.3%), *Clostridia* (from 0.8% to 24%), *Bacilli* (from 0.4% to 9.5%), and *Planctomycetacia* (from 4.5% to 10%) was observed in TR4.

4.2.2.3. Comparison of RO permeates

Compared with TR4, minor changes in the bacterial community composition were observed in TR5 such as the relative increase in the abundance of *Proteobacteria* and *Bacteroidetes* and the decrease of

Planctomycetes as shown in Figure 3. *Actinobacteria* reached their highest abundance during the water production process constituting 10.4% of the total bacterial community in the BWRO permeate. At the class level, the relative abundance of *Clostridia* and *Bacteroidia* increased in TR5 unlike the *Bacilli* and *Planctomycetacia*. After the second RO pass, the *Proteobacteria* community was dominated by the *Alphaproteobacteria*.

4.2.3 Effect of chlorination of produced drinking water

The bacterial community was dominated by *Firmicutes* (49%) followed by *Bacteroidetes* (26%), *Proteobacteria* (10%) and *Synergistetes* (3%). Based on their respective relative abundance, chlorination did not affect *Bacteroidetes*, but reduced the relative abundance of *Actinobacteria* and *Proteobacteria*. At the class level, the relative abundance of *Clostridia* increased from 28% to 44%, while the abundance of *Cytophagia* and *Bacteroidia* decreased from 7.8% to 0.6% and from 26% to 21%, respectively. The *Proteobacterial* community was dominated by members of the *Betaproteobacteria* (45.2%) followed by the *Alphaproteobacteria* (25.2%), *Gammaproteobacteria* (21.5%), *Deltaproteobacteria* (7.2%) and *Zetaproteobacteria* (0.7%).

4.3. RO filtration removed bacterial cells

The conductivity data of the water before and after the SWRO filtration indicated that salt ions were removed strongly (99%). Bacteria are much bigger than salt ions, suggesting that bacteria, also the very small-sized ones in seawater, were rejected by the SWRO. The separation properties of RO membranes are not based on pores (like the membrane types microfiltration, ultrafiltration and nanofiltration) but on diffusion of water through the membranes, indication that there are no pores in the RO membrane that allow bacteria to pass. The bacterial cell numbers in the water after RO filtration were most likely lower than the lower limit of detection (Table 1).

Therefore, RO filtration showed extensive removal of bacteria from the RO feed water. However, the reduction in water DNA concentration (77%) was much less than the reduction in bacterial cell numbers (>99%), possibly due to: i) contamination during water sample collection and processing, ii) presence of free DNA in the RO produced water, iii) intrusion of bacteria that occurred during the

plant start-up (e.g. during placement of the RO membrane modules in the installation) and/or maintenance and inspection work in the RO installation and/or reservoirs, iv) the RO filtration did not eliminate microbial processes in the produced water, and/or (v) a combination thereof. Despite the oligotrophic environment bacterial growth can occur in the RO permeates and the produced drinking water (Park and Hu, 2010).

4.4. Bacterial strains observed at all stages of treatment: not caused by sample contamination

Control measurements to quantify possible contamination of the water samples with DNA from the materials used and the environment (e.g. sample handling, extraction kit, water filtration unit, membrane and air contact) resulted in the detection of unique OTUs affiliated to 16 bacterial species (Table S2 in supplementary material). The bacterial species in the control samples differed from the core bacterial species present in all the different compartments of the desalination plant, indicating that the treatment plant core OTUs were not caused by sample contamination.

4.5. Controls of potential contamination important for clean water studies

Contamination of DNA was found during control studies (Table S2, supplementary material). The DNA contamination can originate from e.g. sample handling, the DNA extraction kit, the water filtration unit, membrane and air contact and combinations thereof. The specific sources of the contamination were not determined.

Contamination could be especially important for environments with “low DNA levels” such as air and clean water. Examples of “low DNA level” environments are the permeates of membrane-based separation processes such as ultrafiltration, nanofiltration and reverse osmosis, as well as industrial and drinking water. Presently, contamination controls are not applied in studies characterizing and evaluating the microbial ecology during drinking water production and distribution.

4.6. Chlorination pre-treatment did not eliminate microbial processes

One of the most serious problems in RO applications is biofouling – excessive growth of biomass – affecting the performance of these membrane systems, influencing the (i) amount and quality of the

produced water and/or (ii) reliability of water production and (iii) costs (Ridgway and Flemming, 1996; Shannon et al., 2008, Vrouwenvelder et al., 2001, 2008, 2011, van Loosdrecht et al., 2012). In the Middle East, about 70% of seawater RO membrane installations suffer from biofouling problems (Gamal Khedr, 2000).

A strategy pursued to prevent and control membrane biofouling is metabolic inactivation of the feed water bacteria by applying chemicals such as chlorine. RO membranes are sensitive to free chlorine. Free chlorine damages the thin-film composite membrane structure causing a decrease in membrane rejection. Therefore, residual chlorine in the seawater has to be removed prior to the RO membranes, which can be achieved by sodium bi-sulphate dosage.

This study shows that the taxonomic composition of the bacterial communities differed between the inlet water (residual chlorine) and after the cartridge filter (after de-chlorination). Of special interest is the increase in the relative abundance of *Cyanobacteria* after the spruce filter, which can be associated with increased numbers of these microorganisms if the total cell counts before and after the spruce filter are considered.

Chlorination prior to the desalination plant is not an effective strategy to inhibit microbial processes and to control membrane biofouling. The change in microbial structure over TR1, TR2 and TR3 illustrated by e.g. the increase in the Beta-Proteobacterial class (Figure 3) and the variation in relative abundance of the non-Proteobacterial community (Figure 5) suggest that there is bacterial growth in the seawater during the SWRO pre-treatment. However, further studies are needed to better characterize this bacterial growth. Dissecting the extent and mechanisms of microbial growth in the different stages of a treatment train requires further investigations.

4.7. Presence of bacteria related to biofouling in desalination plant

Estimation of the degree of biofouling is an important task in RO plant operation and management. Diverse bacterial communities and marine environments can severely affect the SWRO membrane systems (Chun et al., 2012). Therefore, there is a high demand for detailed understanding of the microbial community comprising the biofilm on RO membranes. The 16S rRNA gene pyrosequencing method was successfully used to establish the fouling profile of SWRO membranes

(Kim et al., 2013). Although biofilm formation has been extensively investigated, there are no studies in the literature on variation of the planktonic bacterial communities during desalination for drinking water production. Numerous bacterial genera have been found to participate in biofilm development on RO membranes for fresh or brackish water, among them *Pseudomonas*, *Corynebacterium*, *Flavobacterium*, *Aeromonas* spp. and fungi (Baker and Dudley, 1998). Microbial community analyses of RO membranes employing seawater as the water source and the use of a genetic approach as the identification method are seldom reported.

In this study, 6 bacterial genera known as organisms potentially responsible for biofouling were detected in the water during the desalination process (i.e., *Lactobacillus*, *Corynebacterium*, *Sphingomonas*, *Mycobacterium*, *Flavobacterium*, and *Pseudomonas*). All these bacterial genera were detected specifically in the RO permeates (TR4 and TR5). Distinctively, members of the genera *Pseudomonas* and *Sphingomonas* are known to produce the exopolysaccharides alginate and gellan, respectively (Freitas et al., 2011), and this physiological trait can favor their attachment to surfaces. Both *Flavobacterium* and *Pseudomonas* genera were exclusively detected in TR4 while *Mycobacterium* was only detected in TR5. 1,408 OTUs related to *Sphingomonas* genus were identified in TR4. Members of this genus have been previously reported as initial colonizers in larger fouling layers in a freshwater RO treatment facility and considered responsible for biofouling of RO membranes (Bereschenko et al., 2008). *Lactobacillus*, which was reported as a biofouling cause (Matin et al., 2011), was detected in both SWRO and BWRO permeates in which 299 and 116 related OTUs were identified, respectively. The *Alpha*- and *Gammaproteobacteria* were the most dominant groups of the microbial community detected in the bulk water during the water desalination process, but these bacteria were found to be also dominant members in the biofilm of SWRO membranes (Zhang et al, 2011). *Alphaproteobacteria* were reported to dominate over other bacterial communities in the fouling layer of full-scale SWRO membranes, fed by Red Sea water (Khan et al., 2013).

5. Conclusions

Exploration of the bacterial community structure and variation in water at different treatment stages of a full-scale seawater desalination plant for 40,000 m³/day drinking water production revealed that:

- Chlorination at the seawater intake point was not effective in inhibiting microbial processes in the water treatment train. The taxonomic composition of the bacterial communities differed between the inlet water (residual chlorine) and water after the cartridge filters (after de-chlorination), indicating that the chlorination process was not effective in (i) inactivating all bacteria and (ii) preventing microbial growth in the treatment train, including the RO membrane system.
- Spatial changes in the bacterial community structure reflect the changes in water quality after the different treatment steps.
- As expected,
 - o the two-stage reverse osmosis filtration strongly reduced the water conductivity (>99%), TOC (98.5%) and the total bacterial cell number (>99%).
 - o reverse osmosis and chlorination were the most important processes shaping the bacterial community structure and diversity.
- As not expected,
 - o 0.25% of the total bacterial OTUs was present at all stages of the desalination plant: the seawater, the reverse osmosis permeate and the chlorinated drinking water, suggesting that these bacterial strains can survive in strongly different environments such as high/low salt concentration and with/without residual disinfectant.
 - o control studies evaluating sample filtration and DNA extraction protocols without water showed that DNA contamination occurred, highlighting the importance of controls for studying the ecology of clean water. The bacterial strains found at all stages of treatment (0.25% of total OTUs) did not originate from sample contamination.

Additional investigations are needed to determine the origin of the DNA detected after reverse osmosis and chlorination treatment steps. Biofilm analysis is needed to confirm the presence, locations and the role of biofilms during desalination treatment.

Acknowledgements

This publication is based upon work supported by the KAUST Office of Competitive Research Funds (OCRF) under Award No. URF/1/1728-01-01 and Evides Waterbedrijf. Special thanks to all KAUST desalination plant staff for their technical assistance and to Noredine Ghaffour and Qingyu Li for providing water quality data and discussions.

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Table 1. Water quality parameters and total bacterial cell count during desalination process

Sample ID	Conductivity (mS/cm)	TOC (mg/L)	Residual chlorine (mg/L)	Temperature (°C)	pH	TCC (Cells/mL)
TR1	65.0 (±0.3)	1.68 (±0.06)	< 0.02	25.6 (±0.3)	8.9 (±0.2)	5.47×10^4 (±172)
TR2	61.3 (±0.2)	1.10 (±0.04)	< 0.02	24.1 (±0.1)	8.9 (±0.2)	3.16×10^4 (±93)
TR3	59.7 (±0.1)	0.76 (±0.06)	< 0.02	24.4 (±0.1)	8.1 (±0.1)	2.74×10^4 (±153)
TR4	0.87 (±0.02)	n.d.	< 0.02	24.7 (±0.2)	7.7 (±0.1)	$\leq 200^*$
TR5	0.12 (±0.02)	0.04 (±0.02)	< 0.02	23.7 (±0.4)	10.1 (±0.3)	$\leq 200^*$
TR6	0.22 (±0)	0.02 (±0.00)	0.5	23.5 (±0.2)	7.5 (±0)	$\leq 200^*$

Abbreviations: TR1: inlet seawater, TR2: after spruce media filter, TR3: seawater reverse osmosis feed, TR4: seawater reverse osmosis permeate, TR5: brackish water reverse osmosis permeate and TR6: final chlorinated drinking water. TOC: Total Organic Carbon. TCC: total cell count. *Flow cytometry detection limit. n.d.: not determined; The values between parenthesis represent the standard deviation of three measurements of the same water sample.

Table 2. Alpha diversity indices of bacterial phylotypes

Sample ID	Number of sequences*	Average length (bp)	Observed OTUs	Chao1	Shannon index
TR1	12,945	380	1925	2082	8.59
TR2	50,098	375	3211	2176	7.98
TR3	68,462	375	3781	1641	7.75
TR4	12,065	381	876	445	7.97
TR5	13,364	381	1074	574	7.24
TR6	24,318	382	1761	614	6.08

Abbreviations of the samples are the same as those used in Table 1. The indices were calculated based on the abundance of each operational taxonomic unit (OTU) (97% cutoff). Chao1 is a nonparametric estimation of asymptotic species richness. bp: base pair. * Sequence reads that passed all quality controls (see materials and methods). TR1, inlet seawater; TR2, after spruce media filter; TR3, seawater reverse osmosis feed; TR4, seawater reverse osmosis (SWRO) permeate; TR5, brackish water reverse osmosis (BWRO) permeate and TR6, produced drinking water.

Figure 1. Schematic diagram of the drinking water treatment plant. Sampling sites are indicated with circles as follows: TR1, inlet seawater; TR2, after spruce media filter; TR3, seawater reverse osmosis feed; TR4, seawater reverse osmosis (SWRO) permeate; TR5, brackish water reverse osmosis (BWRO) permeate and TR6, produced drinking water. TR6 is a blend of TR4 and TR5. Dosed are chlorine, antiscalant, SBS (sodium bisulfite), NaOH, CO₂ and lime.

Figure 2. Principal coordinate analysis (PCoA) based on unweighted UniFrac distance matrix showing the phylogenetic relatedness between bacterial communities retrieved from different sampling sites. Abbreviations: TR1, inlet seawater; TR2, after spruce media filter; TR3, seawater reverse osmosis feed; TR4, seawater reverse osmosis permeate; TR5, brackish water reverse osmosis permeate and TR6, produced drinking water.

Figure 3. Taxonomic composition of the bacterial communities revealed by 16S rRNA gene pyrosequencing at the phylum level. Abbreviations: TR1, inlet seawater; TR2, after spruce media filter; TR3, seawater reverse osmosis feed; TR4, seawater reverse osmosis permeate; TR5, brackish water reverse osmosis permeate and TR6, produced drinking water.

Figure 4. Taxonomic distribution of the Alphaproteobacterial (a), Betaproteobacterial (b), Gammaproteobacterial (c), and Deltaproteobacterial (d) orders during water production process. Abbreviations: TR1, inlet seawater; TR2, after spruce media filter; TR3, seawater reverse osmosis feed; TR4, seawater reverse osmosis permeate; TR5, brackish water reverse osmosis permeate and TR6, produced drinking water.

Figure 5. Variation of the non-Proteobacterial community composition during the desalination process. Abbreviations: TR1, inlet seawater; TR2, after spruce media filter; TR3, seawater reverse osmosis feed; TR4, seawater reverse osmosis permeate; TR5, brackish water reverse osmosis permeate and TR6, produced drinking water.

Figure 6. Venn diagrams displaying overlap between the bacterial communities across different sampling sites based on bacterial OTUs (3% distance cutoff); (a) during the pre-treatment step, (b) during reverse osmosis treatment steps and (c) after chlorination. TR1, inlet seawater; TR2, after spruce media filter; TR3, seawater reverse osmosis feed; TR4, seawater reverse osmosis permeate; TR5, brackish water reverse osmosis permeate and TR6, produced drinking water.

Highlights

- Bacterial communities in seawater desalination plant for drinking water production
- High bacterial diversity throughout all explored water treatment stages
- Filtration, RO and chlorination each had a distinct imprint in bacterial community
- Potential sample contamination controls are important for clean water studies