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Impact of blended tap water and desalinated seawater on biofilm stability

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ABSTRACT

Seawater, converted by reverse osmosis (RO) membrane into desalinated water when introduced to drinking water distribution systems and mixed with tap water of natural source, may affect the stability of existing biofilms attached to the pipeline. A continuous flow system consisting of four identical, parallel 1 L CDC biofilm reactors was installed. The reactors were operated with dechlorinated tap water for 55 days. Thereafter, water made of 100% tap water, 100% RO desalinated seawater, and 70/30 and 30/70 mixed tap water/desalinated seawater were continuously applied. Analyses of the bulk water heterotrophic plate count (HPC), biofilm HPC, total carbohydrate content (TCC), and denaturing gradient gel electrophoresis (DGGE) was carried out. No obvious changes in HPC and TCC were observed in bulk water. But, continuous feed with 100% desalinated water resulted in higher bacterial count than the other treatments. The DGGE data showed that higher portion of the RO desalinated seawater resulted in less biodiversity.

Keywords: Biofilm; Desalinated water; HPC; TCC; DGGE

1. Introduction

Biofilm growth on the surface of drinking water pipelines is a well-known phenomenon, particularly under tropical conditions. In drinking water systems, virtually any surface in contact with water will be colonized by microorganisms [1]. It is a natural tendency of microorganisms to attach to wet surfaces, multiply, and embed themselves in a slimy matrix composed of extracellular polymeric substances (EPS) that they produce, forming a biofilm [2]. In coastal areas, seawater desalination is becoming an increasingly important option for viable or even indispensable source of drinking water in many parts of the world as global freshwater sources are becoming increasingly scarce due to rapid population growth and economic expansion [3]. In general, the economic impacts caused by introducing desalinated water to

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existing drinking water supplies greatly depend on the post-treatment. The high production costs of desalination can be justified when combination of water quality modeling was considered [4]. RO-membrane desalinated water, when injected to an existing pipeline and mixed with tap water of natural source, may affect the stability of biofilms attached to the pipeline. There are few studies on the impact of desalinated seawater on biofilms, although there are some studies about the different factors affecting biofilm growth in drinking water distribution systems (DWDS), such as addition of ammonium, phosphorous or nitrate, residual chlorine concentration, flow velocity, and residence time [5–7]. This study was performed to gain a fundamental understanding the effect of mixing desalinated seawater with tap water, on changes in existing biofilms and formation of new ones in DWDS.

2. Materials and methods

2.1. Feed water

Tap water was stored for 48 h in a 10 L tank to allow total residue chlorine to decay to less than the detection limit (0.01 mg/L Cl_2). The dechlorinated tap water was then pumped into the reactors using a peristaltic pump at 1 L/d. At day 55, the feed waters were changed to 100% tap water, 100% desalinated water, and 70/30 and 30/70 (volume portions) mixed tap/desalinated water. The characteristics of tap water and RO desalinated seawater are listed in Table 1.

2.2. CDC biofilm reactor and operational conditions

CDC biofilm reactor (Model CBR 90–1 CDC, Biosurface Technologies, Bozeman, MT, USA) was set up for the study (Fig. 1). Each reactor had eight polypropylene coupon holders suspended under an ultra-high-molecular-weight polyethylene-ported lid. Each coupon holder accommodated three removable coupons. Each coupon had an exposed surface area of 2.53 cm^2 (1.27 cm in diameter). A total of 24 coupons were held in each biofilm reactor. The lid with coupon holders and coupons was mounted in a 1-L Pyrex glass vessel with side-arm discharge port. The glass vessel was placed on a controlled stir plate to provide constant rotation of the baffled stir bar at a fixed speed. Rotation of the baffle provided constant mixing and consistent shear to the coupon surface. The reactor had a working volume of 400 mL.

Throughout the course of this study, the biofilm reactors were run at a fixed rotational speed of 130 rpm and with a hydraulic residence time of 1 day. The flow rate of feed water into the biofilm reactor was 1 L/d. Each reactor was run at a temperature of 28°C with periodic bulk water and biofilm sampling. All reactors were covered with an opaque material to prevent the potential for phototrophic growth in the reactor system.

3. Biofilm sampling and analysis

3.1. Heterotrophic plate count

Standard Method (9215C) for examination of water and biofilm [8] was adopted for heterotrophic plate

Table 1
Characteristics of tap water and RO desalinated sea water

Water parameter	Unit	Tap water	RO desalinated seawater
Turbidity	NTU	<3	<3
Total alkalinity	mg/L as $CaCO_3$	25	3.6
Total hardness	mg/L as $CaCO_3$	55.0	3.2
Total organic carbon (TOC)	mg/L	0.4395	0.0545
Calcium	mg/L	14.69	0.23
Magnesium	mg/L	1.10	0.46
Sodium	mg/L	4.92	16.53
pH		7–9	7.25
Redox potential	mv	−50.2	−12.4
EC	uS/cm	116.05	95.60
Total dissolved solids	mg/L	66	47
Dissolved oxygen	mg/L	3.10	6.29
Chloride	mg/L	14.95	25.38
Sulfates	mg/L	15.46	Not detectable (n.d.)
Phosphates	mg/L	Not detectable (n.d.)	Not detectable (n.d.)
NO_3 (Nitrate)	mg/L	n.d.	0.36
NO_2 (Nitrite)	mg/L	n.d.	0.16

Fig. 1. Experiment set up.

count (HPC). HPC was obtained by spread plating with appropriate dilutions of water samples on the R2A medium. Water samples for HPC were taken every five days via the outlet port. Biofilm samples for HPC were removed by ultrasonication every five days. Duplicate plates were incubated at 30°C for five days before enumeration of colonies. The results were expressed as number of colony forming units (CFU) per ml and CFU per cm^2.

3.2. Total carbohydrate content

Total carbohydrate content (TCC) analysis or modified phenol-sulfuric acid method was used for the measurement of EPS concentrations in the biofilm samples with glucose as the standard. TCC was quantified colorimetrically as described by Dubois et al. [9]. Into the biofilm samples, which had been homogenized and heated in an 80°C oven for 20 min, 1 mL of phenol (90% wt/wt) and 5 mL of concentrated sulfuric acid (97%) were added. The samples were mixed thoroughly and let sit at room temperature for another 30 min. The optical density of resulting yellow-orange color liquid was measured at 490 nm using Hach DR/4000 spectrophotometer (Hach Company, Loveland, CO, USA). The results were expressed in equivalent μg glucose per cm^2.

3.3. DNA extraction, amplification, and characterization with denaturing gradient gel electrophoresis

3.3.1. DNA extraction

Biofilm samples from each reactor were collected for DNA extraction. DNA extraction was performed by using Mobiol Powerwater DNA isolation kit and the extractions followed all specifications in the kit method. The purity and concentration was determined by NANO drop (Bio frontier, USA). The extracted samples were used for polymerase chain reaction (PCR) amplification.

3.3.2. Primers and PCR amplification

The 16S rDNA of the extracted samples was amplified by PCR using bacterial primers, GC-clamp-EUB f933 and EUB r1387, which are specific for universally conserved bacterial 16S rDNA sequences [10]. The sequences of the two primers are EUBf933: 5′-GCA-CAAGCGGTGGAGCATGTGG-3′ and EUBr1387: 5′-GCCCGGGAACGTATTCACCG-3′. Hot-start PCR was performed at 95°C for 10 min. Touchdown PCR was then as follows: the annealing temperature was initially set at 65°C and then decreased by 0.5°C each cycle until it was 55°C. Twenty additional amplifying cycles were run at 55°C. Denaturation was carried out at 94°C for 1 min and primer extension was performed at 72°C for 3 min. The final extension step was at 72°C for 7 min. PCR products were detected by flashgel system (Lonza, USA).

3.3.3. DGGE

DGGE was performed on a Dcodek system from Bio-Rad. UPPCR 16S rDNA fragments were loaded onto 8% (wt/vol) polyacrylamide gel, which was made with a denaturing gradient ranging from 40 to 65%. The denaturant (100%) contained 7 M urea and 40% formamide. Electrophoresis was run in 1X TAE running buffer at 60°C for 12 h at 100 V [11]. After electrophoresis, the gels were stained for 30 min with SYBR Gold nucleic acid gel stain (Molecular Probes), as specified by the manufacturer, and then detected by Fluor-Sk Multi Imager (Bio Rad, USA).

4. Results and discussion

4.1. HPC in bulk water and biofilm

The reactors, each with 400 mL water, were fed with dechlorinated tap water for 55 days. As HPC bacteria were significantly correlated with the quantity of free and total chlorine [7], in the biofilm experiment using tap water, the total residue chlorine was reduced to less than the detection limit (0.01 mg/L Cl_2). Thereafter, 100% desalinated water and mixed tap/desalinated water in volume portions of 70/30 and 30/70 were continuously applied to three of the four reactors. For comparison, the remaining reactor

was fed with dechlorinated tap water during the entire experimental period. The HPC of bulk water (planktonic HPC) in the reactors and biofilm grown on PVC coupons in the reactors were periodically collected for 100 days and analyzed for concerned parameters separately.

The bulk water HPC and biofilm HPC grew on the coupons in each of the reactors are shown in Figs. 2 and 3, respectively. As can be seen from Fig. 2, the planktonic bacteria growth stabilized at 10^4 cfu/ml after 15 days. This level is similar with those reported previously in other studies about tap water [5–7].

After water quality changed on day 55 to 100% desalinated water or the mixture of tap/desalinated water, planktonic cell counts dropped a little and then recovered quickly and there was no apparent changes as the HPC for all reactors remained at similar level in the rest of the running period. However, the HPC of biofilm with 100% RO desalinated water was a little higher than those for treatments of mixed water (Fig. 3).

The results indicated that HPC of both the bulk water and the biofilm samples did not decrease after the introduction of 100% desalinated water and mixed tap/desalinated water at the two ratios. The existing biofilm did not recede as a result of the decrease in TOC concentration, when the RO water was introduced which had a TOC concentration of 0.0545 mg/L, while that of the tap water was 0.4395 mg/L. Instead, the HPC of biofilm with 100% RO desalinated water was a little higher than those for treatments of mixed water. In our previous study [12], using lime and CO_2 as the remineralized method, we also found that remineralized desalinated water stimulated more bacterial growth compared to tap water. It is suspected that TOC from surface of polypropylene materials could be released into the reactor and this could be the reason that HPC of biofilm with 100% RO desalinated water was a little higher than those for treatments of mixed water. A similar phenomenon was found in a study conducted by Likanen et al. [13] showing that the microbial growth in the NF permeate waters was stronger than that in the feed water. In that study, they used the raw water from a small humus-rich lake, and the TOC contents in the NF permeate waters were generally less than 0.3 mg/L, whereas the TOC in feed water was 2.17 mg/L.

4.2. TCC level in biofilm

Polysaccharide is a major component of EPS [2,14]. The production of EPS is essential for biofilm formation as EPS serves as the main "cement" for cells and cell products, traps nutrients, and protects cells [14]. It provides cells a shelter from environmental stress such as high salinity, extreme pH, UV radiation, and desiccation and thus permits organisms survival under hostile conditions [15]. High polysaccharide content has been shown to facilitate cell-to-cell adhesion and strengthen the biofilm structure through a polymeric matrix [16,17]. In order to confirm the data obtained by direct enumeration on R2A agar, the evolution of the biofilm TCC in the four reactors were determined as a function of running time (Fig. 4). The TCC stabilized at 3.0 μg/cm^2 after 30 days. After the RO desalinated seawater was introduced at day 55, no obvious changes in TCC were observed in the four reactors.

4.3. Bacteria diversity in biofilm

The DGGE fingerprinting of the PCR products of biofilm microorganisms amplified with GC-clamp-EUB f933 and EUB r1387 was shown in Fig. 5. PCR

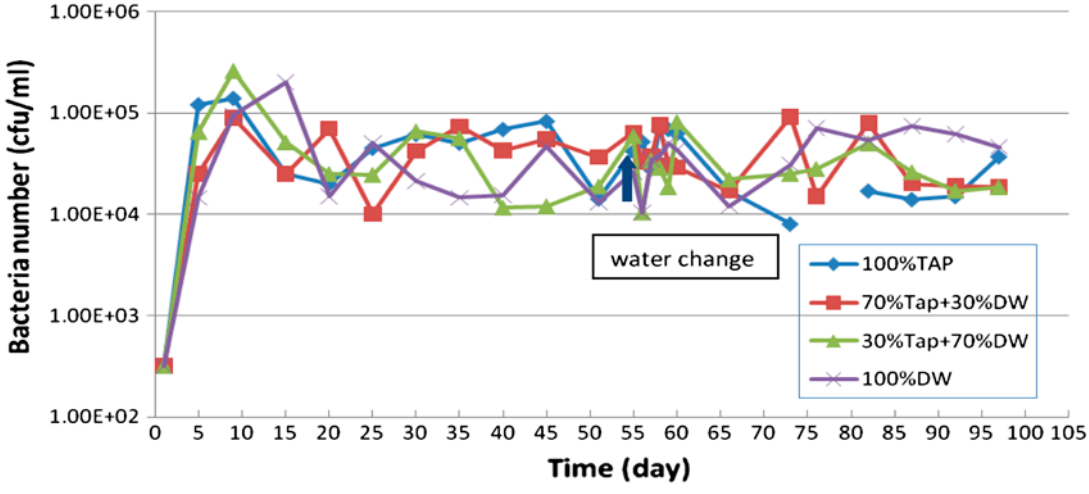

Fig. 2. The HPC of bulk water from different treatments (TAP, tap water; DW, RO desalinated seawater).

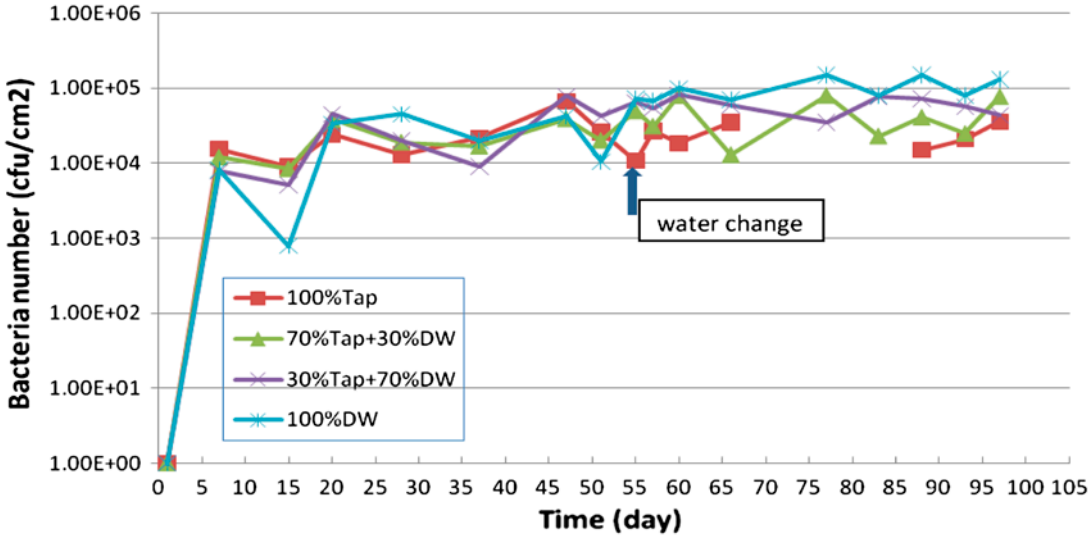

Fig. 3. The HPC of biofilm developed on coupons from different treatments (TAP, tap water; DW, RO desalinated seawater).

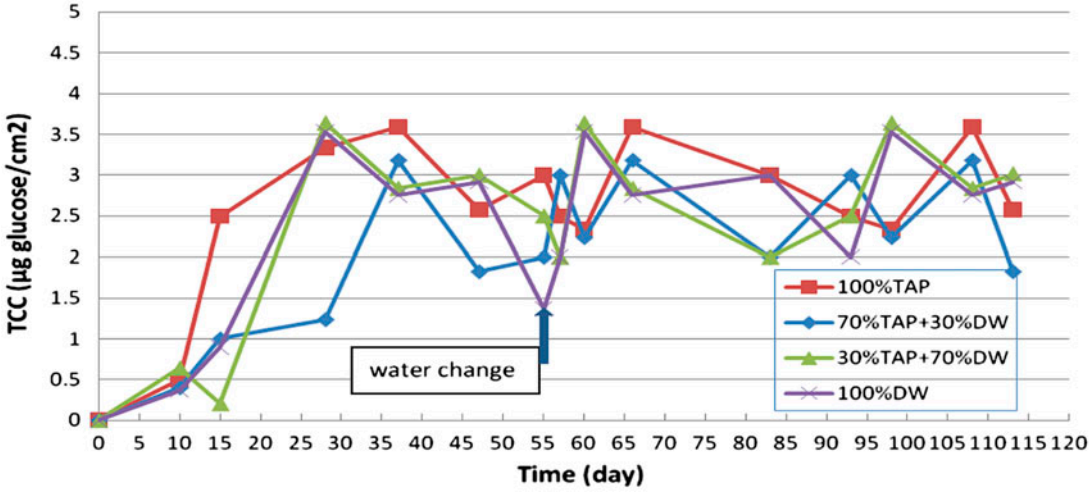

Fig. 4. TCCs from different treatments (TAP, tap water; DW, RO desalinated seawater).

products were loaded on the lanes. Lanes R1, R2, R3, and R4 showed the DGGE pattern for biofilm of 100% tap water, 70/30 and 30/70 tap/desalinated water ratios and 100% desalinated water, respectively, after running the reactors for 80 days. Comparing the bands of biofilm from the four reactors, it can be seen very clearly that R1–R3 has more bands than R4, although the HPC data showed that the bacterial count was almost the same for all reactors. As can be seen from the figure, bands 2, 3, 4, 5, 9, 10, 11, and 12 were found in the biofilm of all reactors, however, bands 6, 7, and 8 were found in R1–R3 but disappeared in R4. There is an indication that biodiversity decreases with RO water addition to Singapore water supply systems, while biofilm growth may persist.

It was very obvious that bands 2 and 3 were stronger in R1, than that in R3 and R4. Band 9 was much weaker in R4 than in other reactors and band 5 was more intense in R3 than in other reactors. Band 12 was more intense in R3 and R4 than in R1 and R2. Since the quantity of species was represented by the band intensity in DGGE patterns, it indicates that species represented by bands 2 and 3 become less in R3 and R4 than R1 and R2, and species represented by bands 5 and 12 were proliferated in R3 and R4 than R1 and R2. It can thus be concluded that some species

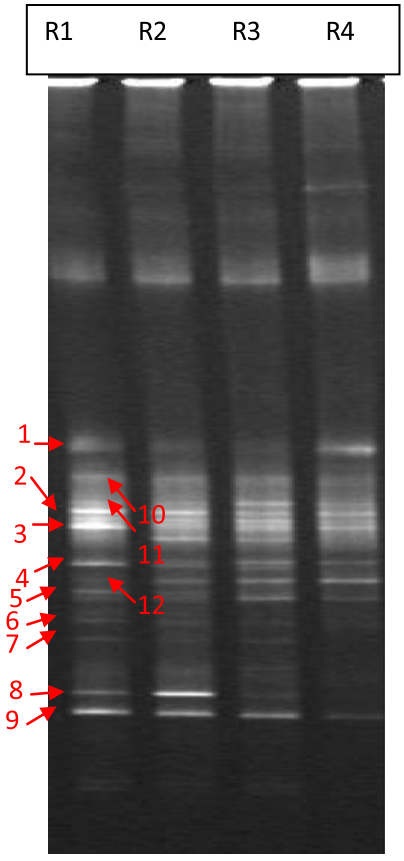

Fig. 5. Bacteria diversity in biofilm grown in water with different mixings (R1: 100% tap water, R2 and R3: mixed tap water/desalinated seawater at portions of 70/30 and 30/70 and R4: 100% desalinated water).

became less and even disappeared when the water was gradually changed to desalinated water. Some other species were however stimulated and proliferated in desalinated water than in tap water.

5. Conclusion

Results presented in this study suggested that changing the feed water from normal tap water to different ratios of tap/desalinated water did not affect the planktonic and biofilm HPC very much, but affected the bacterial population in both the biofilm and the bulk water. HPC of both bulk water and biofilm samples were not suppressed by the introduction of RO membrane desalinated seawater, except while the biodiversity might have been reduced in the water distribution systems. The organisms in the biofilm with the introduction of 100% desalinated water could even grow to an extent slightly more than that in the tap water.

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