



Changes in the relative abundance of biofilm-forming bacteria by conventional sand-filtration and microfiltration as pretreatments for seawater reverse osmosis desalination

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ABSTRACT

A pilot-plant to desalinate seawater using reverse osmosis membrane has been in operation for 1.7 years. Two different pretreatment systems, the conventional process with sand-filtration and the membrane-based process of microfiltration with 0.6 μm pore size, have been used. Besides the physical, chemical, and economic indices needed to evaluate the efficiency of the pretreatment systems, the microbial community structure should be analyzed in order to ensure the removal of bio-foulants, marine biofilm-forming bacteria. In this study, the removal of biofilm-forming bacteria by two seawater reverse osmosis (SWRO) pretreatment systems was qualitatively evaluated through the construction of a 16S rRNA gene library. The relative abundance of certain member of γ -proteobacteria, which are responsible for the major pioneering activity in the formation of biofilms by adhesion to surfaces in the marine environment, was calculated. Raw seawater was dominated by biofilm-forming bacteria of *Alteromonas* (39.2%), *Cowellia* (7.6%), and *Glaciecola* (43.0%) genera. The bacterial diversity was significantly changed by the conventional pretreatment system, while microfiltration had little effect on the diversity. The conventional pretreatment system maintained the dominance of biofilm-forming bacteria, but the sum of the relative abundance of biofilm-forming bacteria was decreased from 79.8% to 50.0%. By decreasing the dominance to only 10.0%, microfiltration showed an efficiency superior to that of the conventional pretreatment system for the removal of biofilm-forming bacteria.

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1. Introduction

Commercially available desalination technologies, including multiple-effect distillation (MED), multi-stage flash distillation (MSF), vapor compression (VC), reverse osmosis (RO), and electrodialysis (ED) have become important sources of potable water. Among these technologies, RO offers high quality water for drinking and industrial purposes, reduction of plant size, and simple water treatment processes [1]. For the production of drinking water using RO processes, surface, brackish, and seawater are utilized. Due to the limited availability of fresh and brackish water, seawater reverse osmosis (SWRO) plants have been widely implemented, nowadays. RO membranes are able to remove most organic and inorganic compounds and microorganisms from the raw water. To ensure high quality of purification, the salt rejection capability of an SWRO process is an important factor and previous studies of SWRO have reported enhanced salt rejections over 99% [2,3].

Despite breakthroughs in SWRO technology, susceptibility to fouling is a major problem of SWRO processes imposing serious performance

limitations and low performance reliability due to the presence of colloidal, particulate, and dissolved organic and inorganic matter in feed seawater. The pretreatment mitigates membrane fouling and ensures improved performance of SWRO. Several pretreatment options are available for SWRO. Conventional technologies include an open seawater intake, screens for coarse prefiltration, and chemical additions (break-point chlorination, acid addition, in-line coagulation, and addition of a flocculation aid) followed by a single- or double-stage sand-filtration [4–7]. Otherwise, non-conventional technologies are based on the membrane technologies of microfiltration (MF), ultrafiltration (UF), and nanofiltration (NF) [8–11]. Recently, membrane based pretreatments have been increasingly considered for utilization to replace less efficient conventional pretreatment systems [7].

Until now, pretreatment performance has been evaluated based on physical indices such as turbidity, total suspended solids (TSS), silt density index (SDI), and particle distribution; on chemical indices of dissolved organic carbon (DOC) and natural organic matter (NOM); and on economic indices of capital and operational costs. Besides physical, chemical, and economic factors, the microbial community structure is one of the critical parameters for the performance of the pretreatment systems because marine biofilm-forming bacteria lead to serious problems in SWRO processes. For example, biofilms in SWRO induce

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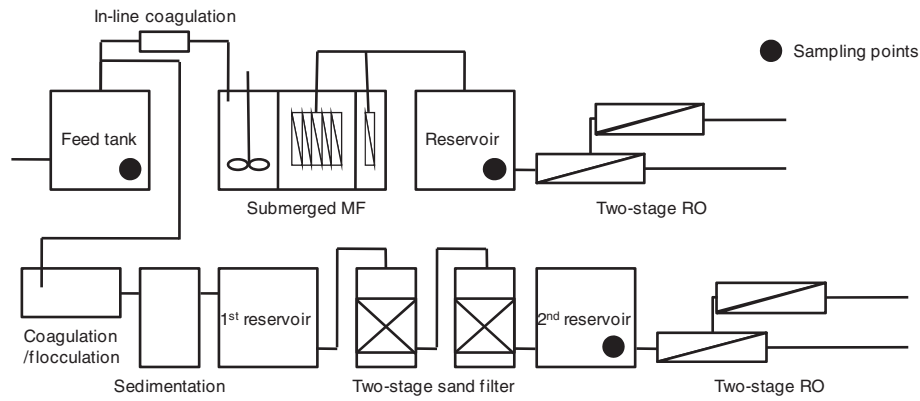


Fig. 1. Schematic diagram of seawater reverse osmosis process with a conventional pretreatment system and with membrane-based pretreatment.

reduced flux, reduced energy efficiency, differential pressure increments, membrane biodegradation, and lowered silt rejection efficiency [12]. However, there has been no research to analyze microbial community structure to evaluate biofilm-forming potential of permeates from the pretreatment systems for SWRO.

The main aim of this study was to analyze the microbial community structure in raw seawater (RS) and permeates from the conventional pretreatment system (P-CP) and microfiltration (P-MF), especially focusing on the relative abundance of putative biofilm-forming bacteria, which have been identified in previous research. For the rapid and convenient identification of biofilm-forming bacteria, culture-independent tools such as clone library, real-time quantitative PCR, fluorescence *in-situ* hybridization (FISH), and microarray are required because the majority of environmental microorganisms are not culturable. Thus, in this study, clone libraries of 16S rRNA gene sequences retrieved from RS and of the permeates from pretreatment systems were constructed by using bacterial universal primers. The bacterial community structure of raw seawater (RS) was first analyzed for fundamental information and then bacterial community structures of the permeates from the two pretreatment systems were compared to that of RS to monitor the changes in microbial community structures and biofilm-forming potentials.

2. Materials and methods

2.1. Operation of seawater reverse osmosis process

The schematic diagram of an SWRO desalination pilot-scale plant is shown in Fig. 1. The plant which treats 200 L/m² h was consisted of conventional pretreatment and microfiltration systems with an SWRO

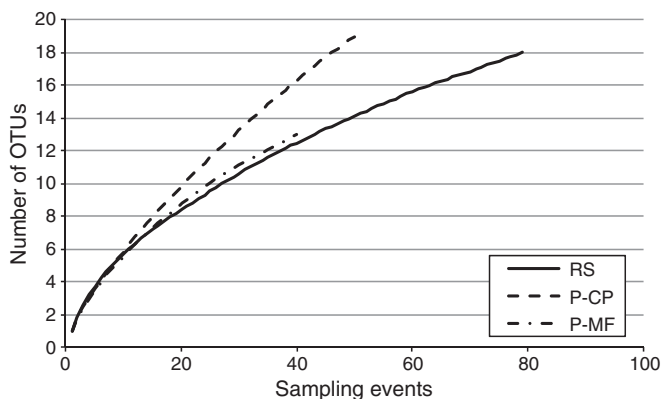


Fig. 2. Rarefaction curves generated by Mothur analysis with 16S rRNA gene sequences sampled from raw seawater (RS) and from permeates of the conventional pretreatment system (P-CP) and microfiltration system (P-MF) using the 3% cut-off.

process. The raw feed water was dosed with FeCl₃ coagulant at 0 to 8 mg/L for both treatment systems. The conventional pretreatment system of coagulation, settlement, and filtration and the membrane-based pretreatment system of microfiltration have been in operation for 1.7 years. The average temperature of the raw seawater during the operation was 14.4 ± 6.5 °C and that for the month before sampling was 20.4 ± 1.2 °C in June and July. The average turbidity of raw seawater was 3.6 ± 2.9 NTU and it was soared in rainy season up to about 20 NTU because seawater was mixed with run-off at the coastal area near the pilot-plant. The feed water for the SWRO was taken from an open intake site in South Korea. Raw seawater and permeates from a sand-filtration at the end of the conventional pretreatment system and from a membrane filtration system with 0.6 μm pore size were sampled. With the conventional pretreatment system, turbidity was decreased by 92.9 ± 0.1%. Microfiltration showed a higher removal of turbidity of 97.3 ± 0.04%.

2.2. DNA extraction and amplification

2 L of raw seawater and permeates of the microfiltration and the sand-filtration systems were filtered through a cellulose acetate membrane filter (47 mm diameter, 0.2 μm pore size) to collect microorganisms. The filter was cut into small pieces with a sterilized scissors; the next step was to proceed to DNA extraction using a Power Soil™ DNA kit (Mo Bio Laboratories, US). The 16S rRNA gene of bacteria was amplified with primer pairs of 27 F (5'-AGAGTTTGATC(A/C) TGGCTCAG-3') and 1492R (5'-GGTACCTTTGTTACGACTT-3'). The PCR mixture, a total of 20 μL, consisted of 13 μL of Taq polymerase PCR premix (Solgent, Korea), 1 μL of forward primer (10 μM), 1 μL of reverse primer (10 μM), and 5 μL of template DNA. The PCR cycles consisted of the following parameters: 1 cycle of 5 min at 94 °C; 30 cycles of 60 s at 94 °C, 45 s at 58 °C, and 90 s at 72 °C; and then 1 cycle of 10 min at 72 °C to extend the reaction using the MyCycler™ Thermal Cycler (Bio-Rad, US).

Table 1
16S rRNA gene diversity analysis in this study.

Sample	Sequences (n)	OTU (97%)	Chao	ACE
RS	79	28	25.2 (19.6–50.4) ^a	31.1 (21.4–68.5)
P-CP	50	27	34.6 (23.2–76.5)	87.7 (51.6–163.5)
P-MF	40	18	22.2 (16.6–47.4)	22.4 (19.2–78.9)

RS: raw seawater, P-CP: permeate from conventional pretreatment system, P-MF: permeate from membrane-filtration system.

All indices were calculated using the MOTHUR package, version 1.7.2

Chao: Chao 1 richness estimate, ACE: ACE richness estimate.

^a The value for the lower and upper bound on the 95% confidence intervals are indicated in the parentheses.

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