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DOI

[10.1016/j.desal.2019.06.001](https://doi.org/10.1016/j.desal.2019.06.001)

Publication date

2019

Document Version

Final published version

Published in

Desalination

Citation (APA)

Abushaban, M., Salinas-Rodriguez, S. G., Dhakal, N., Schippers, J. C., & Kennedy, M. D. (2019). Assessing pretreatment and seawater reverse osmosis performance using an ATP-based bacterial growth potential method. *Desalination*, 467, 210-218. <https://doi.org/10.1016/j.desal.2019.06.001>

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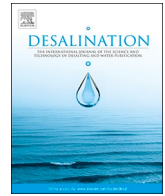
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Assessing pretreatment and seawater reverse osmosis performance using an ATP-based bacterial growth potential method

Almotasembellah Abushaban^{a,b,*}, Sergio G. Salinas-Rodriguez^a, Nirajan Dhakal^a,
Jan C. Schippers^a, Maria D. Kennedy^{a,b}

^a IHE Delft Institute for Water Education, Environmental Engineering and Water Technology Department, Westvest 7, AX, 2611 Delft, Netherlands

^b Delft University of Technology, Faculty of Civil Engineering and Geosciences, Stevinweg 1, CN, 2628 Delft, Netherlands

ARTICLE INFO

Keywords:

Seawater reverse osmosis
Biofouling
Assimilable organic carbon
Bacterial growth potential
Adenosine triphosphate

ABSTRACT

Various bacterial growth potential (BGP) methods have been developed recently to monitor biofouling in seawater reverse osmosis (SWRO) systems such as assimilable organic carbon and bacterial regrowth potential. However, the relationship between these methods and biofouling in SWRO desalination plants has not yet been demonstrated. In this research, an attempt is made to investigate if a correlation exists between BGP of SWRO feed water and the chemical cleaning frequency in SWRO plants using an ATP-based BGP method employing an indigenous microbial consortium. Using ATP-based BGP method at 5 different seawater locations showed low variations of bacterial yield.

The BGP method was applied to assess the pretreatment performance of three full-scale SWRO plants with different pretreatment processes. Dual media filtration (DMF) showed the highest BGP removal (> 50%) in two SWRO plants. Removal of BGP and hydrophilic organic carbon in dissolved air floatation combined with ultrafiltration was similar to the removal achieved with DMF in combination with inline coagulation. For the three SWRO plants investigated, a higher BGP in SWRO feed water corresponded to a higher chemical cleaning frequency. However, more data is required to confirm if a real correlation exists between BGP and biofouling in SWRO plants.

1. Introduction

Biofilm formation on reverse osmosis (RO) membrane surfaces is inevitable [1] and may cause biofouling in some cases. Biofouling occurs when biofilm formation is excessive to the extent that operational problems arise [2]. To monitor biofouling in full scale RO plants, head loss is commonly monitored across the first stage of the RO. Once head loss increases to a significant level (usually about 15% increase from the initial head loss), membrane cleaning-in-place (CIP) is applied to maintain the desired permeability. The frequency of cleaning primarily depends on the biofouling potential of the feed water and the operational conditions (flux, pressure, concentration polarization and CIP

efficiency) of SWRO [3,4].

An early warning system to predict biofouling potential seems more suitable than taking action after pressure drop/head loss has increased [5,6]. Early warning systems may allow optimization of RO pretreatment processes. However, to date, there are no methods or tools available that can predict biofouling in membrane-based desalination systems. The membrane fouling simulator (MFS) and biofilm formation rate (BFR) can be used to monitor biofilm development on a membrane surface, but the biofilm formation in these systems/takes almost the same amount of time needed for biofilm formation on a RO membrane surface [7].

Recently, the use of growth potential methods has gained high

Abbreviations: AOC, Assimilable organic carbon; ASW, Artificial seawater; ATP, Adenosine triphosphate; BDOC, Biodegradable dissolved organic carbon; BFR, Biofilm formation rate; BGP, Bacterial growth potential; BPP, Biomass production potential; BRP, Bacterial regrowth potential; CDOC, hydrophilic dissolved organic carbon; COD, Chemical oxygen demand; DAF, Dissolved air flotation; DMF, Dual media filtration; DOC, Dissolved organic carbon; EC, Electrical conductivity; FCM, Flow cytometry; LC-OCD, Liquid chromatography – organic carbon detection; LMW-A, Low molecular weight acid; LMW-N, Low molecular weight neutral; LOD, Limit of detection; MFS, Membrane fouling simulator; NOM, Natural organic matter; R², Regression coefficient; RO, Reverse osmosis; SWRO, Sea water reverse osmosis; TOC, Total organic carbon; UF, Ultrafiltration; UV, Ultraviolet absorbance

* Corresponding author at: IHE Delft Institute for Water Education, Environmental Engineering and Water Technology Department, Westvest 7, AX, 2611 Delft, Netherlands.

E-mail address: motasem.abushaban@gmail.com (A. Abushaban).

<https://doi.org/10.1016/j.desal.2019.06.001>

Received 11 February 2019; Received in revised form 15 April 2019; Accepted 1 June 2019

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interest among researchers as they may be directly linked to biofilm formation on RO membrane [8–10]. These methods include assimilable organic carbon (AOC) [11], bacterial regrowth potential (BRP) [7,12], and biomass production potential (BPP) [13,14]. The relationship between these methods and biofouling development in full-scale plants has not yet been determined. In fresh water, Hijnen et al. [15] reported that 1 µg/L of AOC (as acetate) added to MFS feed water led to a significant pressure drop within 3 months. Weinrich et al., [16] tested the biofouling of 30 and 1000 µg-C/L on a bench-scale SWRO membrane and reported higher fouling on the RO membrane surface with 1000 µg-C/L (as acetate) than with 30 µg-C/L in RO feed.

The AOC method was initially developed for freshwater by Van der Kooij et al., [11] and was measured by pasteurizing the sample (at 70 °C for 30 min), inoculating it with *Pseudomonas fluorescens* P17 bacteria, incubating it over time (for 2 weeks) and measuring bacterial growth using plate counting. In this method, one pure strain (*Pseudomonas fluorescens* P17) is used, which cannot completely assimilate AOC due to its lack of *exo*-enzymes and interactions between different bacteria. *Spirillum* sp. NOX (NOX) was later added together with P17 by Van der Kooij and Hijnen to utilize oxalic acid for bacterial growth [17,18]. Although these two strains (P17 and NOX) utilize a wide range of easily biodegradable compounds, they cannot utilize more complex compounds such as polysaccharides and proteins. Sack et al. [19] introduced an additional bacterial culture (*Flavobacterium johnsoniae* strain A3) to the freshwater AOC test which target polysaccharides and proteins as nutrients for growth.

Another approach is the use of an indigenous microbial consortium to further broaden and diversify the substrate utilization range in comparison to a single pure culture. Ross et al. [20] demonstrated that bacterial growth using an indigenous microbial consortium was higher (> 20%) than bacterial growth of pure strains and provides a more realistic interpretation of growth potential in water. Several AOC methods have been developed using an indigenous microbial consortium for freshwater based on microbial adenosine triphosphate (ATP) [13], turbidity [21], and total cell counts [22].

Reporting growth potential-based methods as an AOC concentration is questionable as the calibration is performed using only one carbon source (glucose or acetate), while in real water, AOC is a mixture of different carbon sources. To overcome this problem, Withers and Drikas [12] developed a turbidity-based BRP method to monitor bacterial growth in water distribution systems employing the typical procedure of the AOC method in which bacterial growth is reported as µg-C/L (acetate equivalent). Moreover, Van der Kooij and Veenendaal [14] developed the BPP method for drinking water in which the maximum bacterial growth and the cumulative biomass production are reported (in ng-ATP/L) without a conversion to carbon concentration (in µg-C/L).

In seawater, two AOC methods have been developed recently to measure the growth potential in the pretreatment and in the feed of a SWRO membrane system by Weinrich et al. [23] and Jeong et al. [8] using a single strain of bacteria (*Vibrio fischeri* and *Vibrio harveyi*, respectively). The use of a single bacterial strain allows normalization of the yield based on a carbon source, enabling conversion of bacterial growth to a carbon concentration. However, this method may not reflect the carbon utilization of indigenous microorganisms in seawater, and thus it may underestimate the nutrient concentration in seawater. In addition to the two AOC methods, Dixon et al. [7] used a turbidity-based BRP method (developed by Withers and Drikas [12]) to evaluate SWRO biofouling using an indigenous microbial consortium. Table 1 summarizes the available growth potential methods that can be applied in seawater.

The bacterial enumeration method employed to monitor growth potential depends on the bacterial culture. Conventional enumeration methods (i.e., heterotrophic plate counting, total direct cell count) are labourious, time consuming and limited to a small percentage of the overall bacterial count [28]. Weinrich et al. [23] and Jeong et al. [8]

Table 1
Growth potential methods that can be applied in seawater [7,8,23–27].

Reference	Bacterial inactivation	Culture	Enumeration method	Incubation temperature	Expressed results
Weinrich et al. (2011) [23]	Pasteurization (70 °C for 30 min)	<i>Vibrio fischeri</i>	Bioluminescence	30 °C	µg-C as acetate equivalent
Dixon et al. (2012) [7]	Filtration (0.2 µm)	Indigenous microorganisms	Turbidity	Not available	µg-C as acetate equivalent
Jeong et al. (2013) [8]	Pasteurization (70 °C for 30 min)	<i>Vibrio harveyi</i>	Bioluminescence	25 °C	µg-C as glucose equivalent
Quek et al. (2015) [27]	–	Indigenous microorganisms	Microbial electrolysis cell biosensor	20 °C	µg-C as acetate equivalent
Abushaban et al. (2018) [25]	Pasteurization (70 °C for 30 min)	Indigenous microorganisms	Microbial ATP	30 °C	µg-C as glucose equivalent
Dhakal et al. (2017) [26]	Filtration (0.22 µm)	Indigenous microorganisms	Intact cell counts by FCM	30 °C	µg-C as glucose equivalent
Farhat et al. (2018) [24]	Filtration (0.2 µm)	Indigenous microorganisms	Total ATP and total cell count by FCM	30 °C	µg-C as acetate equivalent

used bioluminescence to monitor bacterial growth, as both methods employ luminescent bacteria (*Vibrio fischeri* and *Vibrio harveyi*, respectively). Due to the lack of fast and accurate bacterial enumeration methods, Dixon et al. [7] and Quek et al. [27] used turbidity and microbial electrolysis cell biosensor, respectively, to measure bacterial growth potential in seawater using an indigenous microbial consortium. Recently, new alternative methods that are fast, reliable, accurate and culture-independent have been developed in seawater with low level of detection, such as flow cytometry (FCM) [24,26,29] and ATP [24,25,30].

The removal of bacterial growth potential along SWRO pretreatment trains has been discussed in the recent literature using the newly developed methods. Weinrich et al. [23] reported high variations (20–70%) in AOC removal through a sand filter (Tampa Bay desalination plant) and 50% AOC removal (from 20 to 10 µg C-acetate/L) in ultrafiltration (0.01 and 0.04 µm pore size) (Monterey Bay desalination plant). Moreover, Weinrich et al. [31] reported 43% removal of AOC in the media filtration with inline coagulation (0.6 mg-Fe³⁺/L) at the Al Zawahra desalination plant, UAE. This is similar to the reported removal by Abushaban et al. [30], in which 44 and 7% removal of bacterial growth potential were observed through seawater glass media filtration (without coagulation) and ultrafiltration, respectively, in a pilot plant in the Netherlands. Whereas, Jeong et al. [32] found insignificant AOC removal (4%) through dual media filtration (DMF) combined with inline coagulation (ferric sulphate, dose is not mentioned) due to continuous dosage of sodium hypochlorite to the seawater intake of Perth SWRO desalination plant. Weinrich et al. [31] studied the removal of AOC in three SWRO plants and reported higher AOC concentration in the SWRO feed due to chemical additions which may increase biofouling potential. The reported AOC in RO feed water ranged between 10 and 180 µg C/L as acetate. Thus, a preliminary AOC threshold of 50 µg C/L as acetate was suggested using growth kinetics and maximum yield of *Vibrio harveyi* bacteria in the saltwater applied in a pilot plant.

In this article, bacterial growth potential (BGP) is measured based on microbial ATP and using an indigenous microbial consortium. Using an indigenous microbial consortium and microbial ATP as an enumeration method may provide more accurate and representative information of bacterial growth in seawater. The BGP was monitored in raw seawater from the North Sea and measured along the pretreatment of three full-scale SWRO desalination plants. Finally, an attempt was made to investigate if any correlation exists between BGP in SWRO feed water and the cleaning frequency (CIP) in SWRO plants based on three full scale SWRO desalination plants.

2. Materials and methods

2.1. Cleaning of glassware

All vials and caps were washed with a lab detergent (Alconox Ultrasonic Cleaner, Alconox, USA), rinsed with Milli-Q water (Milli-Q® water Optimized purification, 18.2 MΩ·cm at 25 °C, EC < 10 µS/cm, TOC < 30 µg/L, Millipore, USA) and submerged in 0.2M HOCl (Merck, Millipore, USA) for 15 h. Afterwards, they were rinsed again three times with Milli-Q water and were air dried. To eliminate potential organic contamination, the vials were heated in a furnace oven for 6 h at 550 °C while the vial caps were bathed in a sodium persulfate solution (100 g/L, Merck, Millipore, USA) for 1 h at 60 °C. Finally, the caps were rinsed with Milli-Q water and air dried.

2.2. Bacterial growth potential measurements

Measuring BGP in seawater comprises four steps, including bacterial inactivation, bacterial inoculation, incubation and bacterial enumeration (Fig. 1). Each step has been studied comprehensively (see supplementary data). Bacterial inactivation and inoculation were used as the

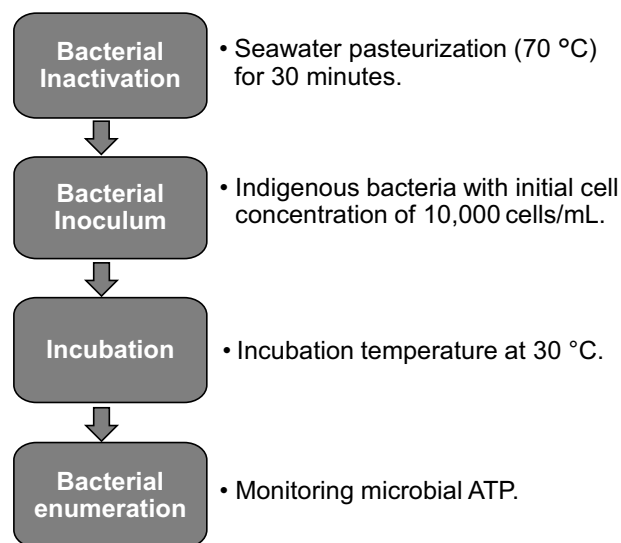


Fig. 1. Procedure of measuring BGP in seawater based on microbial ATP.

microbial population during SWRO pretreatment is not constant in terms of number and composition. Microbial inactivation allows the standardization of the initial microbial population by adding a constant inoculum concentration. Based on results shown in Section S1, both filtration and pasteurization can be used to inactivate the microbial population in seawater. However, due to the possibility of carbon release from virgin filters [26], pasteurization was used. Moreover, sterilization was not used due to the possibility of carbon degradation at a high temperature (Section S1). The heating temperature during pasteurization was also tested, and it was found that there was no carbon degradation when seawater was heated at temperatures between 70 and 100 °C (Section S1). An inoculum concentration (100–20,000 cells/mL) was tested and 10,000 cells/mL was used to ensure sufficient cells for growth and to shorten the growth time to 2 days (Section S2), which agrees with the reported concentration in the literature [22]. Negligible nutrient concentration (< 3%) was estimated to be introduced into the seawater sample from the inoculum (Section S2.1). However, the incubation temperature has a significant effect on bacterial growth; the highest bacterial growth of indigenous microorganisms was achieved when the incubation temperature was similar to the original inoculum temperature (Section S3). This effect was overcome by using a calibration line for carbon and BGP at a constant incubation temperature for each seawater type. Using a calibration line and calculating bacterial yield allows the BGPs of different seawater samples at different locations to be compared.

2.3. Microbial ATP measurements in seawater

Microbial ATP was determined using the direct ATP method for seawater as described in Abushaban et al. [25]. Briefly, total ATP and free ATP were measured to determine microbial ATP (microbial ATP = total ATP – free ATP). For the total ATP measurement, 100 µL of Water-Glo lysis reagent (Water-Glo kit, Promega Corp., USA) was added to 100 µL of the seawater sample in a 1.5 mL Eppendorf tube. The mixture (seawater and lysis reagent) and the Water-Glo detection reagent (Water-Glo kit, Promega Corp., USA) were heated at 38 °C for 4 min. An aliquot of 200 µL of the heated ATP detection reagent was added to the mixture. For the free ATP measurement, 200 µL of pre-heated (at 38 °C for 4 min) Water-Glo detection reagent was added to 100 µL of pre-heated seawater sample in a 1.5 mL Eppendorf tube. The bioluminescence signal was measured using a Promega GloMax®-20/20 luminometer. The measured bioluminescence signals were converted to the total ATP and free ATP concentrations based on 2 calibration curves.

2.4. Bacterial yield

To investigate the bacterial yield in North seawater, bacterial growth with different glucose concentrations (0, 10, 25, 50, 75 and 100 µg-C/L) was monitored (based on microbial ATP) in both real seawater (North Sea, The Netherlands) and artificial seawater (ASW) since the behaviour of indigenous microorganisms in artificial seawater could be different due to the presence of different substrate in real seawater. A correlation was established between the maximum bacterial growth (as ng-ATP/L) and the added glucose concentration. The bacterial yields in seawater and ASW were investigated based on the slope of the correlation line.

Glucose is used in this research as a carbon source as several literature references stated that glucose is a likely substance for assimilation in seawater and concentrations of 10^{-6} – 10^{-8} M glucose are known to be present in seawater [33–35]. Moreover, Weinrich et al. [23] reported higher bacterial growth of marine microorganisms with glucose concentration (0–140 µg-C/L as glucose) than acetate.

2.5. The limit of detection of the ATP-based BGP method

The limit of detection (LOD) of the BGP method was determined using a microbial inoculum from the North Sea in 10 blanks in triplicate, in which ASW (TOC < 30 µg/L) was used as a blank. ASW was prepared as described in Abushaban et al. [25]. Nitrogen (20 µg-N/L as NaNO₃) and phosphorous (5 µg-P/L as NaH₂PO₄·2H₂O) were added to the blank to avoid bacterial growth inhibition due to Nitrogen and Phosphorous limitation. Bacterial growth was monitored based on microbial ATP (LOD = 0.3 ng-ATP/L) [25]. The maximum bacterial growth within 5 days (14.7 ± 1.6 ng-ATP/L) was used as BGP. LOD of BGP (19.5 ng-ATP/L, 13 µg-C/L as glucose) was determined using the following equation [36].

$$\text{LOD} = \text{Average of 10 blanks} + 3 \times \text{standard deviation of 10 blanks}$$

2.6. Monitoring BGP of the North Seawater

BGP, algal cell concentration and water temperature were monitored from the North Sea at the Jacobahaven pilot plant (Kamperland, Netherlands) from January 2016 to January 2017. Raw seawater samples were collected weekly in sterile 500 mL amber-colour glass sampling bottles and transported (90 km) to Delft (Netherlands) in a cooler box (5 °C). The summary of the properties of the collected samples is as follows: total organic carbon (TOC) = 1.28 ± 0.85 mg/L, total cell concentration measured by flow cytometry = 0.9 ± 0.28 × 10⁶ cells/mL, pH = 8.0 ± 0.1 and EC = 52.6 ± 1.2 mS/cm.

2.7. Organic carbon and biopolymer measurement

Liquid chromatography - Organic Carbon Detection was used to measure the hydrophilic organic carbon and biopolymer concentrations. The measurement and analysis of the samples were performed according to the protocol described by Huber et al. [37]. Seawater samples were shipped in a cooler box (5 °C) to Doc-labor Huber lab (Karlsruhe, Germany) for analysis.

2.8. Monitoring BGP along the pretreatment of three SWRO plants

BGP was measured along the pretreatment trains of three large (capacity > 120,000 m³/day) SWRO desalination plants located in the Middle East and Australia. The raw seawater of the three SWRO plants comes from open intakes, in which plant A and plant B have similar characteristics of raw seawater properties (Table 2). The SWRO pretreatment of the three plants are different. Fig. 2 shows the treatment schemes and the locations of all collected samples. Brief specifications and operating conditions of the three plants are presented in Table 3.

Table 2

The properties of raw seawater of the three SWRO desalination plants.

	Plant A	Plant B	Plant C
Salinity (mS/cm)	69–71	69–71	54–60
TDS (g/L)	49–50	49–50	34–35
pH	8.3–8.6	8.3–8.6	8.1–8.3
Turbidity (NTU)	4–10	4–10	1–2
Water temperature (°C)	22–30	22–30	18–25
DOC (mg-C/L)	1.1 ± 0.1	1.1 ± 0.1	0.9 ± 0.1
Silt density index (%/min)	4.7 ± 0.4	4.7 ± 0.4	4.1 ± 0.3
MFI-UF (s/L ₂)	2050	2050	2150
Chlorophyll a (µg/L)	0.6	0.6	NA
Algal concentration (cell/mL)	600	600	NA

3. Results and discussion

3.1. Bacterial yield of indigenous microbial consortia

The conversion of microbial growth to carbon concentrations is only possible if the bacterial yield is known. For an indigenous microbial consortium, the bacterial yield needs to be determined for each location as it may vary depending on the microorganisms present in the inoculum [10,38]. Bacterial yield can be investigated by determining the correlation between the carbon concentration and BGP for a specific location. Having this correlation also allows BGPs of different seawater samples of different locations to be compared.

Bacterial yields of the indigenous microbial consortium in seawater and ASW were investigated using glucose as a carbon source (Fig. 3). Good correlations in seawater ($R^2 = 0.98$) and ASW ($R^2 = 0.99$) were observed between BGP and the added glucose concentration. The higher intercept point of the real seawater (66.8 ng-ATP/L) compared with the ASW (16 ng-ATP/L) is due to the presence of dissolved organic compounds in the seawater (natural background level). The slope of the correlation line in seawater (1.56 ng-ATP/µg-C as glucose) was slightly (9%) higher than in ASW (1.43 ng-ATP/µg-C as glucose), revealing that the bacterial yield in seawater is greater. The difference in the bacterial yields could be attributed to the loss of some marine bacteria when they are placed in ASW, which is not their natural environment. The use of a different substrate in seawater may provide a higher bacterial yield [39].

Similarly, bacterial yields of the Tasman Sea, Arabian Sea, Persian Gulf and Gulf of Oman were also determined (Table 4) using an indigenous microbial consortium collected on situ at each location. The bacterial yield ranged between 1 and 1.5 ng-ATP/µg-C as glucose. The difference in the bacterial yield is attributed to several reasons, including the bacterial diversity present in the seawater and their activity, the carbon (as glucose) utilization rate and the seawater temperature.

3.2. The limit of detection of the ATP-based BGP method

The average BGP of the blank after inoculation with marine microorganisms was 14.7 ± 1.6 ng-ATP/L. Thus, the LOD of the ATP-based BGP method was calculated based on the average of 10 blanks (measured in triplicate) plus three times the standard deviation (14.7 (avg. of blank) + 3 × 1.6 (standard deviation) = 19.5 ng-ATP/L). The bacterial growth in the blank indicates the presence of low concentrations of carbon, which could be introduced from several factors including the seawater inoculum (~5 ng-ATP/L, see section S2), presence of nutrients in the (analytical grade) salts as well as the Milli-Q water used to make up ASW, and contamination from glassware and the surrounding environment. In this research, the blank was not subtracted from the measured BGP of seawater samples as the origin of the nutrients in the blank is not known. Moreover, nutrient concentrations can vary in time as they originate from multiple sources as mentioned above.

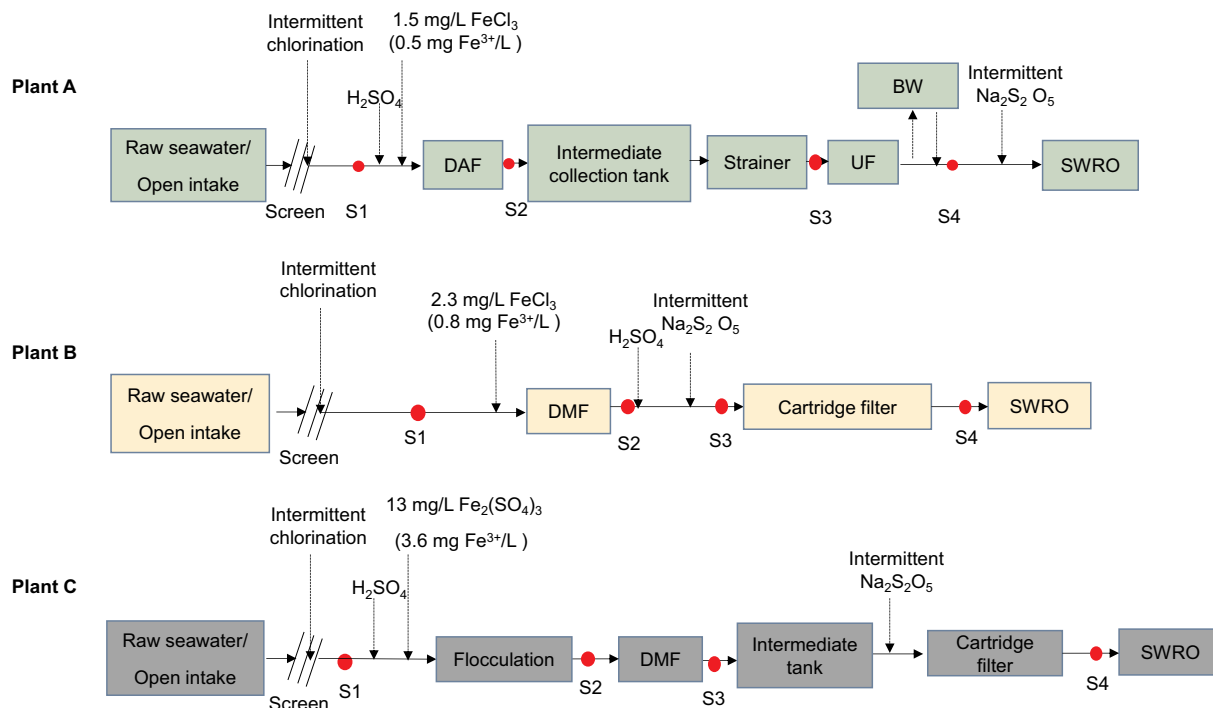


Fig. 2. The treatment schemes of the 3 SWRO desalination plants in the Middle East and Australia, and the locations of collected samples for BGP monitoring.

Table 3
Operating conditions of the three SWRO desalination plants.

	Plant A	Plant B	Plant C
Pre-treatment	Coagulation + dissolved air flotation + ultrafiltration + cartridge filtration	Coagulation + dual media filtration + cartridge filtration	Coagulation + flocculation + dual media filtration + cartridge filtration
pH adjustment	At 7.9 in the intake by H ₂ SO ₄	At 7.4 in the SWRO feed by H ₂ SO ₄	No adjustment
Coagulation dosage (mg-Fe ³⁺ /L)	1.5 mg-FeCl ₃ /L (0.5 mg-Fe ³⁺ /L)	2.3 mg-FeCl ₃ /L (0.8 mg-Fe ³⁺ /L)	13 mg-Fe ₂ (SO ₄) ₃ /L (3.6 mg-Fe ³⁺ /L)
Type of filtration	Vertical ultrafiltration	Pressurized dual media filter	Gravity dual media filter
Type of media		Anthracite and sand	Coal and sand
Depth of media filter		1 m	1.6 m
Filtration cycle	1 h	24–48 h	48 h
Filtration rate (m/h)	0.06 (flux = 60 L/m ² /h)	11–14	10–12
Estimated contact time	< 10 s	4–5 min	8–9 min
Backwash protocol	Water	Air and water	Air and water
Antiscalant dosing	Yes	Yes	Yes
SWRO recovery	40%	40%	40%

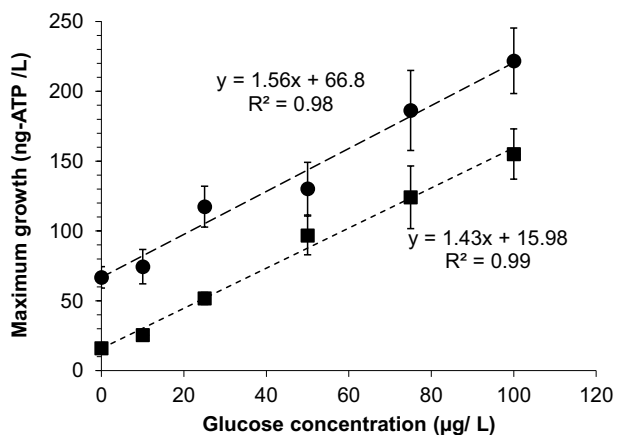


Fig. 3. The correlation between added glucose concentration and the BGP in seawater (●) and artificial seawater (■).

Table 4
The bacterial yields of different microbial consortium of different seawaters.

Source of microbial consortium	Seawater temperature during sampling	Electrical conductivity (mS/cm)	Bacterial yield (ng-ATP/µg-C as glucose)
North Sea	7 °C	52–54	1.5 ± 0.1
North Sea	20 °C	52–54	1.4 ± 0.1
Tasman Sea	25 °C	50–52	1.0 ± 0.1
Arabian Sea	22 °C	54–56	1.3 ± 0.2
Gulf of Oman	30 °C	55–56	1.2 ± 0.2
Persian Gulf	42 °C	69–71	1.3 ± 0.2

Using the investigated bacterial yield of North Sea bacteria in seawater (1.56 ng-ATP/µg-C as glucose) and in ASW (1.43 ng-ATP/µg-C as glucose), the LOD of the BGP (19.5 ng-ATP/L) method was approximately 13 µg-C/L as glucose (19.5/1.5), respectively. Jeong et al. [8] reported 0.1 µg-C/L as glucose of LOD in the AOC method in seawater using *Vibrio fischeri* bacteria. However, the reported LOD was calculated

after subtracting the AOC of the blank, which was $> 50 \mu\text{g-C/L}$ as glucose/L. To convert the LOD to C-acetate, Weinrich et al. [23] found that the glucose utilization by *Vibrio harveyi* bacteria was higher than the acetate utilization at a concentration below $150 \mu\text{g-C/L}$. Assuming the difference in carbon utilization applies to the indigenous microbial consortium as well, the LOD of the BGP method will be $< 10 \mu\text{g}$ acetate/L. This is similar to the reported LOD by Werner and Hamsch [21] and Hammes et al. [40] in freshwater, using an indigenous microbial consortium based on turbidity and total cell counts measured by flow cytometry, respectively. Van der Kooij and Hijnen [17] reported the lowest LOD ($1 \mu\text{g}$ C-acetate/L) of AOC in freshwater, in which plating counts was used to monitor the growth of P17 and NOX.

Lowering the LOD to $< 5 \mu\text{g-C/L}$ as glucose or even below $1 \mu\text{g-C}$ as glucose would be ideal for measuring low BGP in the SWRO feed, particularly, in the winter. However, biofouling is not expected at low water temperatures with a low BGP. In this study, the lowest BGP measured in the SWRO feed was $70 \mu\text{g-C/L}$ as glucose, in which the SWRO membrane was cleaned in place every 3 years (section 3.4) which was > 5 times higher than LOD of the BGP method.

3.3. Monitoring of BGP in the North Seawater

The BGP of raw North Seawater was monitored and a seasonal variation was observed ranging between $45 \mu\text{g-C/L}$ as glucose in the winter to $385 \mu\text{g-C/L}$ as glucose in the spring (Fig. 4). Two seasonal peaks of BGP were obtained in early spring (April) and in autumn (September/October). The BGP and algal cell concentration are similar to the observed trends in dissolved organic carbon (DOC) and chlorophyll *a*, respectively, by Sintes et al. [41] in the coastal North Sea-water. They reported higher DOC values in the spring and autumn and lower DOC values in the winter and high chlorophyll *a* concentration in the spring.

Low algal concentration and BGP were observed at low water temperature ($< 10^\circ\text{C}$, November–February). In March, BGP and algal concentration increased indicating a spring algal bloom. A similar finding was observed by Huck et al. [42], in which a higher AOC concentration was observed in the spring due to algae blooming. However, algal concentration further increased in April and May from 150 to 410 cells/mL while BGP decreased from 350 to $60 \mu\text{g}$ glucose-C/L. The decrease in BGP in the late spring could be attributed to the high nutrient utilization by algae during an algal bloom. Later, in the summer, despite the higher water temperature, algal concentration decreased to very low concentrations (50 cells/mL) while BGP increased to $300 \mu\text{g}$ glucose-C/L. The reduction in algal concentration in the summer could be due to the growth of other microorganisms that

use algae as a source of nutrients (such as Daphnids and Rotifer) [43,44]. Thus, the BGP increased due to low algal concentration thus less competition for nutrients and/or due to released carbon from marine bacteria and algae [45]. The high BGP measured in autumn is consistent with the reported trend by Camper [46], who monitored AOC in 64 surface water treatment plants. LeChevallier et al. [47] monitored AOC and coliforms in 31 full-scale water plants and reported the same trend.

The correlation between BGP, algal cell concentration and water temperature was not evident all the year because both water temperature and the presence of algae influence BGP. For instance, BGP may only correlate with algal concentration during specific seasons (i.e. algal bloom in March/April) since very low algal concentrations were observed during the rest of the year. Moreover, a correlation might be possible between BGP and water temperature when algae does not play a role.

3.4. Monitoring of BGP in three full-scale SWRO desalination plants

3.4.1. Plant A

The SWRO pretreatment of plant A consists of dissolved air flotation (DAF) and ultrafiltration (UF). The measured BGP of the raw seawater (before DAF) was $400 \mu\text{g-C/L}$ as glucose and decreased by 17.5% to $330 \mu\text{g-C/L}$ as glucose after the DAF (Fig. 5a). The organic matter removal through the DAF is lower than that reported in literature. Shutova et al. [48] reported 84, 25 and 16% removal of biopolymers, low molecular weight acids (LMW-A) and DOC, respectively, in a lab scale DAF system fed with Gold Coast seawater with coagulant dose of $3 \text{ mg-Fe}^{3+}/\text{L}$ (at pH 7.5). Whereas, the removal of biopolymers, LMW-A and DOC in the DAF system of plant A was 8, 2 and 2.5%, respectively, (Table 5) using $0.5 \text{ mg-Fe}^{3+}/\text{L}$ coagulant dose at pH 7.9. The low reduction of BGP through DAF could be attributed to the low coagulant dose ($0.5 \text{ mg-Fe}^{3+}/\text{L}$), particularly, at high pH (pH 7.9). It has been reported by Shutova et al. [48] that coagulant dose in seawater DAF depends on pH, in which the optimal coagulation condition for organic matter removal is at low pH. The optimal coagulant dosage is $0.5\text{--}4 \text{ mg-Fe}^{3+}/\text{L}$ at pH 5.5 and $4\text{--}12 \text{ mg-Fe}^{3+}/\text{L}$ at pH 7.5 [48].

A further removal of BGP (32.5%) was observed, mainly in the ultrafiltration (UF) system, where the BGP decreased to $200 \mu\text{g-C/L}$ as glucose. Weinrich et al. [23] reported 50% removal of the AOC concentration (from 20 to $10 \mu\text{g-C/L}$ as acetate) through the ultrafiltration of the Moss Landing desalination pilot plant in California. Whereas, Mathias et al. [49] reported much lower dissolved organic matter removal (20 and 13%) in 50 and 200 kDa seawater lab-scale UF membranes, respectively. The variation in the reported removal of organics

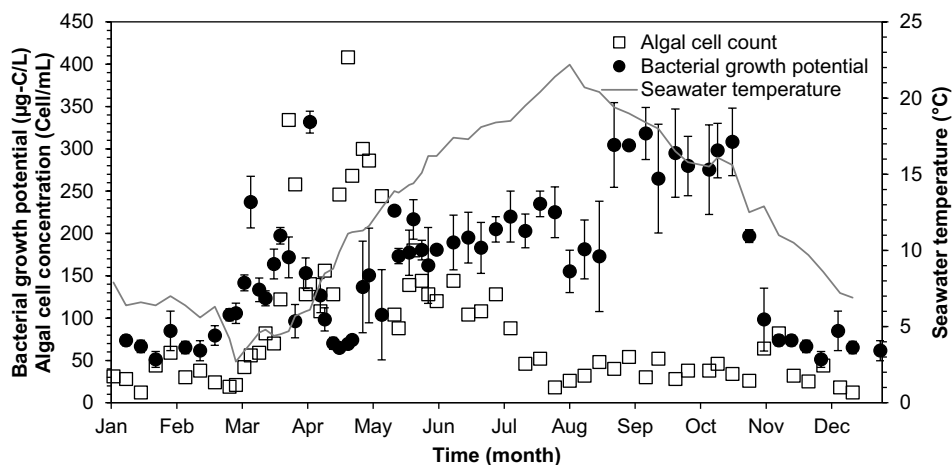


Fig. 4. BGP, algal cell concentration and water temperature throughout 2017 in the North Sea raw seawater at the Jacobahaven pilot plant (Netherlands).

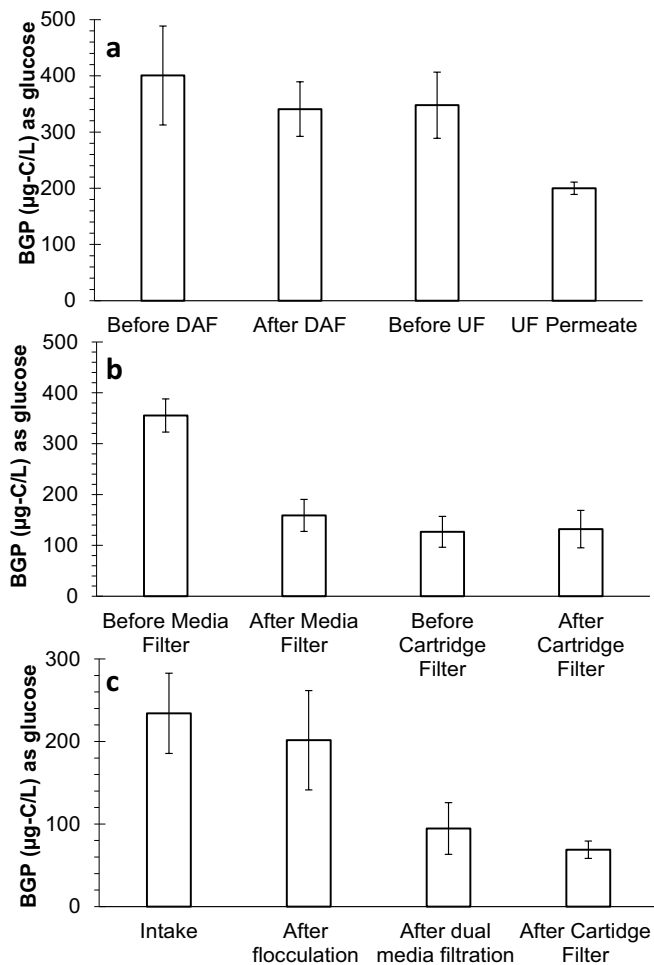


Fig. 5. Monitored BGP in triplicate along the pretreatment trains of three SWRO desalination plants for 3 days (a) plant A in the Middle East, (b) plant B in the Middle East and (c) plant C in Australia.

depends on the type of natural organic matter (NOM) present in the seawater [50]. It can be observed that the SWRO feed of plant A still supports a significant bacterial growth ($> 200 \mu\text{g-C/L}$ as glucose) despite DAF and UF being used as a pretreatment. The total removal of BGP through the pretreatment of plant A was 50%.

Table 5
Comparing the pretreatment and their removal of the three studied SWRO plants.

	Plant A		Plant B	Plant C
	Inline coagulation and DAF	Ultrafiltration	Inline coagulation and pressurized DMF	Coagulation, flocculation and gravity DMF.
Coagulation ($\text{mg-Fe}^{3+}/\text{L}$)	0.5	–	0.8	3.6
Contact time	NA	$< 10 \text{ s}$	4–5 min	8–9 min
BGP removal ($\mu\text{g-C/L}$)	70	130	190	156
BGP removal (%)	17%	33%	55%	68%
CDOC removal ($\mu\text{g/L}$)	27	133	151	NA
CDOC removal (%)	2.5%	12%	15%	NA
Biopolymer removal ($\mu\text{g/L}$)	13	78	35	NA
Biopolymer removal (%)	8%	46%	29%	NA
Humic substances removal ($\mu\text{g/L}$)	10	28	59	NA
Humic substances removal (%)	3%	7%	14%	NA
LMW-N removal ($\mu\text{g/L}$)	11	1	15	NA
LMW-N removal (%)	6.5%	$< 1\%$	9.5%	NA
LMW-A removal ($\mu\text{g/L}$)	2	2	10	NA
LMW-A removal (%)	2%	2%	10%	NA

NA: not available.

3.4.2. Plant B

The pretreatment of plant B consists of single stage pressurized dual media filtration (DMF) after inline coagulation ($0.8 \text{ mg-Fe}^{3+}/\text{L}$). The measured BGP of the seawater before DMF was $350 \mu\text{g-C/L}$ as glucose which decreased to $160 \mu\text{g-C/L}$ after DMF (Fig. 5b). The significant reduction (55%) of BGP through the DMF incorporation with inline coagulation could be attributed to the high biodegradation rate in the DMF and/or the addition of $0.8 \text{ mg-Fe}^{3+}/\text{L}$ coagulation dosage. Similar findings were observed by Weinrich et al. [23] in which the AOC removal through the sand filtration of a Tampa Bay desalination plant ranged between 23 and 80%. BGP after the cartridge filtration (approx. $125 \mu\text{g-C/L}$ as glucose) was similar to the measured BGP after DMF. The overall removal of BGP through the pretreatment processes of plant B was 55%, which was mainly due to coagulation and/or carbon biodegradation in the DMF [51].

3.4.3. Plant C

The pretreatment of plant C is a typical conventional treatment (coagulation, flocculation and gravity media filtration). BGP in the seawater intake was approximately $230 \mu\text{g-C/L}$ as glucose (Fig. 5c), which is the lowest BGP in raw seawater among the three plants (plants A, B and C). Slight removal of BGP (15%) was observed through the flocculation process due to the addition of coagulation with 13 mg/L of $\text{Fe}_2(\text{SO}_4)_3$, equivalent to $3.6 \text{ mg-Fe}^{3+}/\text{L}$. Conversely, a significant removal of BGP (53%) was noted in the DMF. The BGP removal of the conventional pretreatment (coagulation, flocculation and gravity media filtration) of plant C (68%) was higher than the observed BGP removal of the DMF incorporation with inline coagulation of plant B (55%). The higher BGP removal of the conventional pretreatment of plant C could be attributed to the longer contact time in the gravity DMF compared to the pressurized DMF of plant B and/or due to the higher coagulation dosage applied in plant C ($3.6 \text{ mg-Fe}^{3+}/\text{L}$). An insignificant BGP removal (4%) through the cartridge filtration of plant C was found, as expected. The overall BGP removal in plant C was 72%.

3.4.4. Comparing the removal of organic in the three SWRO plants

Comparing the overall removal of BGP and LC-OCD analysis through the pretreatment of plants A and B shows that the combination of inline coagulation and DMF could provide slightly higher removal as that of DAF and UF (Table 5). However, the UF (plant A) showed higher removal of the biopolymers fraction compared to the media filtration of plant B. Poussade et al. [52] compared the removal of UF and media filtration and concluded that the removal rate of dissolved organic matter (expressed as UV_{254} absorbance and TOC) by media filtration was slightly better than that of UF, which was also found in the three SWRO plants studied here (based on BGP and LC-OCD analysis). The

Table 6

Comparing the cleaning frequency and the BGPs of raw seawater and the RO feed of the three SWRO desalination plants.

	Plant A	Plant B	Plant C
BGP of raw seawater, $\mu\text{g-C/L}$ as glucose	400	350	230
BGP of RO feed, $\mu\text{g-C/L}$ as glucose	200	128	70
Overall BGP removal, $\mu\text{g-C/L}$ as glucose (%)	200 (50%)	222 (55%)	160 (72%)
CIP frequency CIP's/year	6	1	0.3

higher removal in media filtration compared to UF could be attributed to the biodegradation in the media filter as the contact time in media filter (4–5 min) is much longer than the contact time in UF (< 10 s). Kim et al. [53] tested the combination of DAF with DMF and found that DAF did not significantly improve the organic removal of DMF. This is also in agreement with the low removal of BGP, CDOD and biopolymer observed through DAF in plant A. It should be noted that low coagulant dosage was added before DAF (plant A) and before DMF (plant B).

The BGP removal through conventional pretreatment (plant C) was comparable to the overall removal achieved in DAF combined with UF (plant A) and inline coagulation incorporated with DMF (plant B). The overall BGP removal through the conventional pretreatment was highest (72%); however, the overall magnitude of the BGP removal (160 μg glucose-C/L) was lower than the removal in the other plants (Table 6). This is mainly because the raw seawater of plant C has a better quality than plants A and B. It should be noted that the coagulant dosage in plant C is very high compared to the applied coagulant dosage in plant B.

By comparing BGP's in the SWRO feed of three desalination plants, it can be seen that plant A has the highest BGP in the feed, while plant C has the lowest (Table 6). This finding indicates that the biofouling potential of plant A is the highest among the three SWRO desalination plants.

Investigating if a correlation exists between BGP in SWRO feed and biofouling in SWRO systems is complicated by several factors. Firstly, several types of fouling (scaling, particulate and organic/biofouling) may occur simultaneously in a SWRO plant. Secondly, to establish a correlation, a large number of SWRO desalination plants in different parts of the world need to be monitored for longer periods of time with different operating conditions. Thirdly, the widespread intermittent use of non-oxidizing biocides to combat biofouling in full scale SWRO facilities makes establishing a real correlation between BGP of SWRO feed water and CIP frequency very difficult.

Despite these limitations, an attempt was made to investigate if a correlation exists between the measured BGP in SWRO feed water and the CIP frequency in the three SWRO plants. The CIP frequency (CIPs per year) was used as a surrogate parameter for biofouling, assuming that scaling and particulate fouling do not occur. This assumption is somehow justified as antiscalant is dosed prior to the SWRO membranes and thus should eliminate the occurrence of any scale. Furthermore, the SDI was always below ($\text{SDI} < 3$) in the SWRO feed water suggesting that particulate fouling was not significant in the SWRO plants studied.

From Table 6, it can be observed that a higher CIP frequency corresponded to a higher BGP of SWRO feed water, suggesting that the BGP method is a promising indicator of biofouling potential in SWRO feed water. However, to establish a real correlation, more data needs to be collected and many more SWRO plants need to be monitored for longer periods of time with different operating conditions. Moreover, the monitoring program should be expanded to include a wide variety of seawater locations and pre-treatment technologies.

4. Conclusion

- A method based on microbial ATP was developed to measure BGP using an indigenous microbial consortium in seawater. BGP was measured in triplicate for 5 days however the maximum growth was reached within 2–3 days.
- The bacterial yield was measured using the ATP-based BGP method in 5 locations and ranged between 1 and 1.5 ng-ATP/ $\mu\text{g-C/L}$ as glucose, thus indicating low variations of the bacterial yield of indigenous microorganisms in terms of microbial ATP. The limit of detection of the BGP method is 13 $\mu\text{g-C/L}$ as glucose.
- BGP of North Sea raw seawater was monitored over a period of 12 months, in which a seasonal variation was observed between 45 $\mu\text{g-C/L}$ as glucose in the winter and 385 $\mu\text{g-C/L}$ as glucose in the spring.
- The method was applied to monitor BGP through the pretreatment trains of three SWRO desalination plants with different pretreatment processes. DMF showed the highest BGP removal (> than 50%) in two SWRO desalination plants and this was attributed to the longer contact time in DMF filters (6 min) compared with UF (< 10 s). The removal of DAF combined with UF was comparable to the removal of DMF in combination with inline coagulation (0.8 mg- Fe^{3+}/L).
- A higher CIP frequency of the SWRO's corresponded to a higher BGP in SWRO feed water, suggesting that the BGP method is a promising indicator of biofouling potential in SWRO feed water. However, to establish a real correlation, more data needs to be collected and many more SWRO plants need to be monitored for longer period of time and with different operating conditions, and the monitoring program should be expanded to include a wide variety of seawater locations and pre-treatment technologies.
- Ongoing research will focus on BGP monitoring in several full scale SWRO plants for longer period of time (6 months) at several different locations.

Acknowledgement

We thank Promega (Madison, USA) for providing ATP Water-Glo reagents and financially supporting this research. Special thanks are due to Nasir Mangal and Chidiebere Nnebuo for their assistance in the preliminary work of this research.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.desal.2019.06.001>.

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