Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2017.21783

99 (2017) 91–101
December

Direct measurement of ATP in seawater and application of ATP to monitor bacterial growth potential in SWRO pre-treatment systems

Almotasembellah Abushaban[a,b,*], M. Nasir Mangal[a], Sergio G. Salinas-Rodriguez[a], Chidiebere Nnebuo[a], Subhanjan Mondal[c], Said A. Goueli[c,d], Jan C. Schippers[a], Maria D. Kennedy[a,b]

[a]*UNESCO-IHE Institute for Water Education, Environmental Engineering and Water Technology Department, Westvest 7, 2611 AX Delft, The Netherlands; emails: s.salinas@un-ihe.org (S.G. Salinas-Rodriguez), m.kennedy@un-ihe.org (M.D. Kennedy)*
[b]*Delft University of Technology, Stevinweg 1, 2628 CN Delft, The Netherlands*
[c]*Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711, USA*
[d]*University of Wisconsin School of Medicine, Madison, WI, USA*

Received 28 September 2017; Accepted 12 November 2017

ABSTRACT

The use of adenosine triphosphate (ATP) to monitor bacterial growth potential of seawater is currently not possible as ATP cannot be accurately measured at low concentration in seawater using commercially available luciferase-based ATP detection. The limitation is due to interference of salt with the luciferin–luciferase reaction, which inhibits light production. This research demonstrates that new reagents developed for (i) ATP extraction from microbial cells and (ii) ATP detection in seawater are able to reliably detect Microbial ATP as low as 0.3 ng L^{-1} in seawater. The luminescence signal of the new detection reagent is significantly higher (>20 times) than the luminescence signal of the freshwater reagent, when applied in seawater. ATP can now be used to monitor bacterial growth potential (BGP) through pre-treatment trains of seawater reverse osmosis (SWRO) plants. The level of detection of the new BGP test is significantly lower than the estimated threshold value required to prevent biofouling in SWRO systems. The new reagents have been used to monitor Microbial ATP in coastal North Sea water. Moreover, Microbial ATP has been applied to monitor the bacterial growth potential (using indigenous bacteria) through the pre-treatment train of an SWRO desalination plant. A significant reduction (>55%) of the bacterial growth potential was found through the dual media filtration with 4.5 mg-Fe(III) L^{-1} coagulant. Overall, the new reagents can detect low Microbial ATP concentrations in seawater and can be used to monitor bacterial growth potential in seawater desalination plants.

Keywords: Adenosine triphosphate; Seawater; Bacterial growth potential; Reverse osmosis; Desalination; Biofouling

1. Introduction

Controlling biological fouling in seawater reverse osmosis (SWRO) membranes at an early stage is key to the successful and cost-effective operation of membrane-based desalination plants. Biofouling of SWRO membranes occurs due to the accumulation of biofilm on the membrane surface, or accumulation across the spacer-filled membrane feed channels to such an extent that the operational problem threshold is exceeded, typically a 15% reduction in initial performance. Operational issues may include an increase in pressure drop across the elements, an increase in salt passage and membrane degradation. To mitigate most of these problems, plant operators clean the membranes as frequently as

* Corresponding author.

the biofouling threshold is exceeded. The cleaning in place (CIP) is performed by soaking and flushing the membrane channels with various chemicals in an attempt to remove the biofilm. The frequency of CIP is site specific and varies from time to time, depending mainly on the biofouling potential of the seawater source, operational conditions and the effectiveness of the pre-treatment processes in removing readily available nutrients.

To date, no single parameter is available that can predict biofouling in membrane-based desalination systems. Biomass quantification is only used as a first indication of biofouling potential [1,2] as bacteria are always present in reverse osmosis (RO) feedwater even after ultrafiltration pre-treatment [3]. Moreover, biofilm formation in RO is inevitable if the feedwater supports significant bacterial growth due to the presence of dissolved nutrients. Hence, bacterial growth potential of RO feedwater has gained more attention than the removal of bacteria itself [4,5]. Several methods directly linked to bacterial growth have been developed such as assimilable organic carbon (AOC) [6], biodegradable dissolved organic carbon [7] and biomass production potential [8,9]. Weinrich et al. [10] detected more biofouling (using a flat sheet RO membrane) when the AOC concentration of the feedwater increased from 30 to 1,000 μg L^{-1}. In addition, differential pressure increased from 3.5 to 6.2 bar during 9 d of pilot testing when the median AOC was 50 μg L^{-1}.

AOC measurements have been widely studied for potential applications involving freshwater employing heterotrophic plate count (HPC) [6,11–13], flow cytometry (FCM) [14], adenosine triphosphate (ATP) measurement [8,15] and bioluminescence [16]. Most of these AOC methods used a pure strain of bacteria as inoculum, and the first AOC method using indigenous bacteria in freshwater was developed by Stanfield and Jago [8] and ATP was used for bacterial enumeration. Ross et al. [17] reported higher (>20%) bacterial growth in freshwater when using indigenous bacteria compared with a pure strain. Similar AOC studies for seawater have lagged behind compared with freshwater. However, Weinrich et al. [18] adapted the AOC-bioluminescence freshwater method for seawater by using a specific strain of bioluminescent marine bacteria, *Vibrio harveyi*. Jeong et al. [4] found a strong correlation between the number of another single bioluminescent strain, *Vibrio fischeri* and the bioluminescence signal (in the range of 10^3–10^5 CFU). Consequently, the bioluminescence of a single strain of bacteria has been increasingly adopted for AOC measurement in seawater. These two methods are fast (1 h and 1–3 d, respectively) but use a pure strain of a single bacterium which may not reflect the carbon utilization of a natural bacterial community in seawater. It should be noted that these two methods cannot be applied with indigenous bacteria because not all naturally occurring bacteria show bioluminescence. Developing an AOC method using indigenous bacteria for seawater (similar to the Stanfield and Jago [8] method for freshwater) may provide results with more predictive value in terms of biofouling in SWRO than the use of a pure bacterial strain.

Several methods can be used in seawater to monitor bacterial growth including HPC, total direct count (TDC) by microscope, FCM and ATP. HPC is laborious, time consuming and limited to the enumeration of cultivable bacteria [19,20]. TDC does not distinguish between active and inactive cells and is limited to samples that have high cell concentrations (>10^7 cell mL^{-1}) [21]. FCM is fast, accurate, and can differentiate between intact and dead cells, nevertheless, it is recommended as a relative method because of the use of a manual gate to distinguish the bacterial cells from other microorganisms, particles and the background of the machine [22,23].

ATP is known as the "energy currency" of cells [24,25] as it is present in all living cells and rapidly degrades when cells die [26]. Thus, ATP is directly related to the activity of biomass [26–28]. ATP in a given water sample can be classified into two separate fractions: Microbial ATP and Free ATP. Microbial ATP is present within the living cellular population in the sample. Free ATP is present outside the cell, which can be generated from the release of cellular ATP upon cell death. ATP has been used to assess microbial activity in drinking water, groundwater, biofilms in distribution networks and to monitor freshwater treatment processes [2,29,30]. In freshwater RO systems, ATP has been applied as a biomass parameter: (i) to quantify biomass on membrane surfaces and diagnose biofouling [1,2], (ii) to measure biomass in the feedwater [31] and (iii) as a biomass parameter in bacterial growth potential measurements [8].

There are no commercially available ATP kits for seawater due to interference from salts. The high ionic strength of seawater has been demonstrated to cause substantial inhibition of the enzymatic ATP reaction, so that the emitted light signal interferes with the background luminescence [32,33]. van der Kooij in Amy [32] suggested diluting seawater with demineralized water to an electrical conductivity of 4 mS cm^{-1} (2.5 mg L^{-1}) to avoid salts interference. However, diluting seawater also substantially lowers the biomass concentration (ATP) which in turn limits the use of the method to samples with high biomass concentrations. Moreover, bacterial cells may burst at low electrical conductivity due to the osmotic pressure shock, and consequently only Total ATP can be determined. Due to lack of an ATP method for seawater, an attempt was made by Simon et al. [34] to use BacTiter-Glo reagent (freshwater regent) to measure ATP at the inlet and outlet of a lab scale biofilter for seawater with reported high ATP concentrations. Limit of detection (LOD) using the freshwater reagent kit (BacTiter-Glo) in seawater was investigated in our group to be 50 ng L^{-1} (2×10^5 cell L^{-1}). van Slooten et al. [33] developed a method based on filtration to quantify ATP of large organisms (10–50 μm) present in ballast water. In this method, organisms are concentrated on a 10 μm filter. Thereafter, the filter is placed in a cuvette with sterile Milli-Q water (Millipore) to concentrate the organisms in a small volume of demineralized water. Limitations are that the method is time consuming and exposing the marine organisms to demineralized water may result in bacterial osmotic shock which underestimates the ATP concentration.

The objective of this article is to illustrate the applicability of new reagents (Water-Glo kit) developed by Promega for microbial lysis and ATP detection in seawater. The Microbial ATP measurement in seawater is intended for monitoring of bacterial growth potential in the pre-treatment and feed of SWRO systems using indigenous bacteria.

To establish the target LOD necessary in SWRO systems, the lowest threshold AOC concentration to avoid

biofouling in RO membranes was used. Hijnen et al. [27] reported 1 μg-acetate L^{-1} in RO feedwater as the threshold value to avoid biofouling in freshwater RO membrane systems. AOC concentration was converted into cell concentration using the conversion factor reported by Hammes et al. [35] (1 μg-acetate L^{-1} = 1 × 10^4 cell mL^{-1}). Assuming that this is applicable to seawater, the ideal method would allow the detection of ATP in seawater samples down to 2.5 ng L^{-1} (using a conversion factor of 1 × 10^4 cell mL^{-1} = 2.5 ng L^{-1} reported in this research; Fig. 3(b)).

The following aspects have been investigated and are described in this article:

- Verifying the luminescence signal and stability of the new detection reagents in seawater.
- Testing the efficiency of the new lysis and detection reagents in seawater.
- Testing the effect of seawater pH and iron concentration on the luminescence signal.
- Calibration curve and the LOD of the measurement.
- Monitoring Microbial ATP in raw seawater.
- Measuring Microbial ATP and bacterial growth potential along the pre-treatment train of an SWRO desalination plant using an indigenous bacterial consortium.

2. Materials and methods

2.1. Sample collection, transportation and storage

Coastal seawater samples were collected in Jacobahaven (Kamperland, the Netherlands) between January and December 2016. All samples were collected in sterile 500 mL glass sampling bottles with tight-fitting screw caps and transported for 120 km in a cooler at 5°C for analysis. The characteristics of the tested seawater are shown in Table 1.

2.2. Preparation of artificial seawater

Artificial seawater (ASW) was prepared using Milli-Q water and analytical-grade inorganic salts (Merck, USA) with ion concentrations similar to the average global seawater [36] (23.67 g L^{-1} NaCl, 10.87 g L^{-1} $MgCl_2.6H_2O$, 4.0 g L^{-1} Na_2SO_4, 1.54 g L^{-1} $CaCl_2.2H_2O$, 0.74 g L^{-1} KCl, 0.21 g L^{-1} $NaHCO_3$ and 0.002 g L^{-1} Na_2CO_3). The pH, electrical conductivity and Total ATP of ASW was 8.0 ± 0.1, 52.6 ± 1.2 and <0.05 ng L^{-1}, respectively.

Table 1
Water quality of seawater from Kamperland (the Netherlands, North Sea)

Parameter	Value
pH	7.9 ± 0.1
TDS (g L^{-1})	32.5 ± 0.8
Conductivity (mS cm^{-1})	52.6 ± 1.2
Total bacterial count (cell mL^{-1})[a]	1.2 ± 0.48 × 10^6
TOC (mg-C L^{-1})	1.28 ± 0.85
UV_{254} (cm^{-1})	0.045 ± 0.009

[a]Measured with FCM.

2.3. Measurement of Microbial ATP in seawater

In this method, Microbial ATP was extracted directly by adding ATP Water-Glo lysis reagent (ATP Water-Glo Kit, Promega Corp., USA) to the seawater sample. Both, Total ATP and Free ATP were measured separately to determine the Microbial ATP (Microbial ATP = Total ATP – Free ATP). The manufacturer of the reagents recommends that the volume of seawater sample plus the volume of lysis reagent should be equal to or less than the same volume of water detection reagent.

To measure Total ATP concentration, 100 μL of ATP Water-Glo lysis reagent (Promega Corp.) was added directly to 100 μL of the seawater sample in a 1.5 mL microcentrifuge tube (sterile Eppendorf tube, Sigma-Aldrich). The mixture was heated at 38°C for 5 min. Following the manufacturer's recommendation, an aliquot of 200 μL of preheated ATP Water-Glo detection reagent (Water-Glo, Promega Crop.) was added to the mixture and then the luminescence was recorded using a luminometer (GloMax®-20/20, Promega Corp.). To measure Free ATP concentration, the same procedure was followed, but, without the addition of the ATP Water-Glo lysis reagent. The measured luminescence signal was converted to ng L^{-1} using two different calibration lines; one for Total ATP and the second one for Free ATP. As the solution matrix is different in each case, a separate calibration line is needed. Calibration lines with ATP concentration ranging from 0 to 500 ng L^{-1} were prepared using ATP standard (100 nM, Promega Corp.) and autoclaved seawater. The Free ATP concentration was subtracted from the Total ATP concentration to get the Microbial ATP concentration. All analyses were performed in triplicate.

2.4. Testing the efficiency of lysis and detection reagent

The lysis effectiveness of the reagent in raw seawater was studied in two parts. The first part compares the new Water-Glo lysis reagent with chlorine. The second part investigated the concentration of microbial cells that can be effectively lysed by Water-Glo lysis reagent.

The lysis efficiency of Water-Glo lysis reagent (Water-Glo kit, Promega Corp.) and free chlorine (8 mg-Cl_2 L^{-1}) was compared based on the Microbial ATP concentration of a seawater sample collected from the North Sea (the Netherlands). The sample was filtered over a 0.1 μm filter (sterilized, Millipore) to accumulate the microorganisms on the membrane surface and thereafter the lysis reagent/solution was filtered through the same filter to extract Microbial ATP from the accumulated microorganisms on the membrane surface. The extracted Microbial ATP concentration in the filtrate was then measured according to the Free ATP protocol described in section 2.3.

To prepare the free chlorine solution, sodium hypochlorite (3.5% Cl_2) was diluted (2,500×) in ASW to obtain 8 mg-Cl_2 L^{-1} of free chlorine. The sample was neutralized (5 min contact time) by adding 10 mM $Na_2S_2O_3$ with a 1% (v/v) ratio [37]. The concentration of chlorine was selected based on the findings of Nescerecka et al. [37] where it was observed that a range of free chlorine concentration (5.6–11.2 mg-Cl_2 L^{-1}) could completely extract Microbial ATP without oxidation of ATP molecules in freshwater.

For the second part of the lysis effectiveness study, marine microorganisms present in 1 L of raw seawater collected from North Sea was concentrated in 30 mL by filtering the seawater (1 L) through a 0.2 µm filter using a vacuum pump. The concentrated marine microorganisms were resuspended in 30 mL of the same seawater sample. The intact cell concentration was then measured by FCM and the concentrated sample was diluted in the filtered raw seawater to get a different concentration of microorganism (1.2×10^5 to 1.2×10^7 cell mL^{-1}).

To investigate whether sufficient Water-Glo detection reagent is present for the ATP reaction, Microbial ATP of a seawater sample was measured for the recommended volume ratio of seawater sample to lysis reagent to detection reagent (100:100:200 µL) and compared with two different volume ratios (100:100:100 µL, 100:200:300 µL), including different volumes of detection reagent. As different total volumes and amount of reagents are used, calibration lines of Free ATP and Total ATP were established to determine the Microbial ATP concentration.

2.5. Effect of pH and iron concentration on the luminescence signal

To study the effect of pH, the luminescence signal of ASW at different pHs, ranging from pH 7 to 8.5, was measured. The pH of ASW was adjusted with 0.03 M HCl (37%, Acros Organics) and 0.03 M NaOH (J.T. Baker). The prepared ASW was filtered through 0.1 µm filter (sterilized, Millipore) to remove bacteria that might be introduced during the pH adjustment. Thereafter, the protocol of Total ATP measurement was followed to measure the luminescence signal.

Similarly, to study the effect of iron, the luminescence signal of ASW in the presence of iron concentrations (0, 0.1, 0.3, 0.5, 1, 2, 3, 5 and 10 mg L^{-1}) was measured by following the protocol of Total ATP. To prepare different concentrations of iron, a stock solution with 2 M $FeCl_3.6H_2O$ (Merck Millipore) was prepared using ASW.

2.6. Monitoring of ATP and bacterial growth in an SWRO desalination plant

Microbial ATP and bacterial growth potential (based on Microbial ATP) were monitored in an SWRO desalination plant in Australia. The RO pre-treatment processes include a drum screen, coagulation and flocculation, dual media filter (DMF) and cartridge filter. Four samples were collected in October 2016 (spring season) through the RO pre-treatment (Fig. 1); just before coagulation (raw seawater), after coagulation and flocculation, after DMF and after cartridge filter.

For bacterial growth monitoring, the samples were pasteurized (for 1 h) and 15 mL was transferred into 30 mL AOC-free vials (heated in an oven furnace for 6 h at 550°C) in triplicate. In order to broaden the bacterial versatility, a natural consortium of a bacterial population from the same location (as the sample) was inoculated (~200 µL inoculum volume) with an initial bacterial cell density of 1×10^4 intact-cell mL^{-1} (measured by FCM) in each vial. The samples were incubated at 30°C. The bacterial growth of the seawater sample was monitored for 5 d using the ATP protocol described in section 2.3.

2.7. Determination of limit of detection

The LOD was determined for both Total ATP and Free ATP based on an average of 10 blanks plus three times the standard deviation of the blank [38]. The LOD of the Microbial ATP method was calculated using the combined procedure, which is the square root of the sum of the squares of Free ATP and Total ATP $\text{LOD} = \sqrt{\text{LOD of Free ATP}^2 + \text{LOD of Total ATP}^2}$.

3. Results and discussion

3.1. Luminescence signal and stability of the new reagents in seawater

The luminescence signal and stability of ATP Water-Glo lysis and detection reagents were tested in seawater and compared with BacTiter-Glo (combined freshwater reagent). The ATP Water-Glo reagent showed higher luminescence signal (>20×) compared with that obtained with BacTiter-Glo, when applied in seawater (Fig. 2(a)). ATP Water-Glo reagent showed a good correlation between ATP concentration and luminescence signal with an R^2 of 0.99. It was also noted that the luminescence background of the ATP Water-Glo reagent is much lower (515 RLU) compared with that of BacTiter-Glo

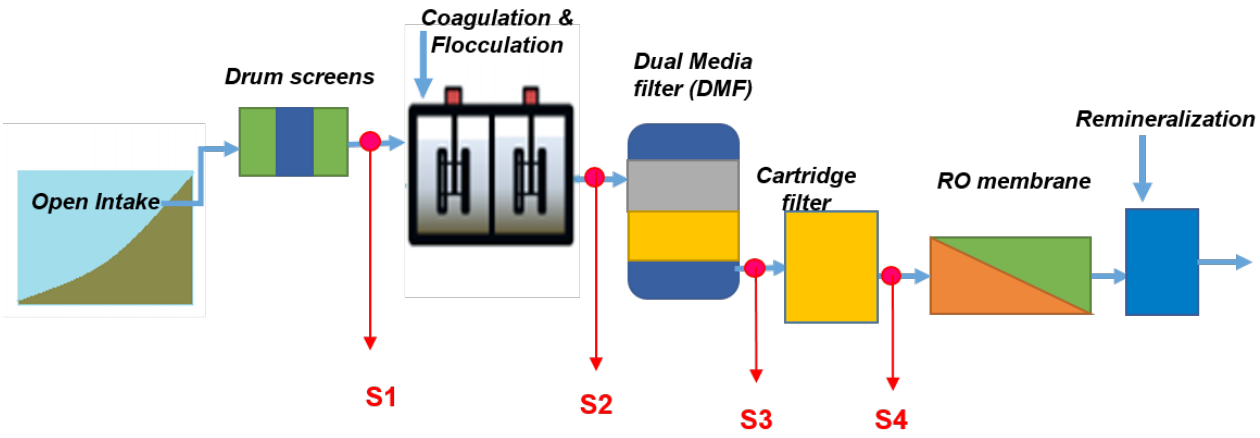

Fig. 1. The treatment scheme of the tested SWRO desalination plant in Australia and the locations of the collected samples.

(2,263 RLU) in artificial sweater (35 g L^{-1}). The high luminescence signal and low background luminescence of the ATP Water-Glo reagents suggest that the new reagents provides more sensitivity than BacTiter-Glo (freshwater reagent) when used in seawater.

A thermostable firefly luciferase is used in formulating the ATP Water-Glo reagent. The ATP Water-Glo reagent is provided as a lyophilized substrate containing a mixture of luciferase and luciferin and a reconstitution buffer. Upon reconstitution, the stability of the liquid reagents was tested when stored at 4°C and 23°C and then compared with the stability of BacTiter-Glo at 23°C. The ATP Water-Glo reagent retained over 90% of its activity for 1 month at 4°C and for 10 d at 23°C, whereas the activity of BacTiter-Glo dramatically decreased within the first day (Fig. 2(b)). The stability of the luminescence signal was also tested, and was stable for 40 s after the addition of ATP Water-Glo reagent to the seawater sample (Fig. 2(c)). These results demonstrate that the new reagents are suitable for application for seawater and are more stable than the existing freshwater reagent when used in seawater.

3.2. Effectiveness of the new lysis and detection reagents

3.2.1. Lysis reagent

The effectiveness of the ATP Water-Glo lysis reagent was tested and compared with the effectiveness of chlorination (8 mg L^{-1}) with respect to cell lysis (Fig. 3(a)). The measured Microbial ATP concentrations when using the ATP water-Glo lysis reagent (120 ng L^{-1}) and 8 mg L^{-1} free chlorine (115 ng L^{-1}) were very similar indicating that the lysis efficiency of Water-Glo lysis reagent is highly effective. A strong lysis reagent may lyse algal cells as well as bacterial cells. However, this has no

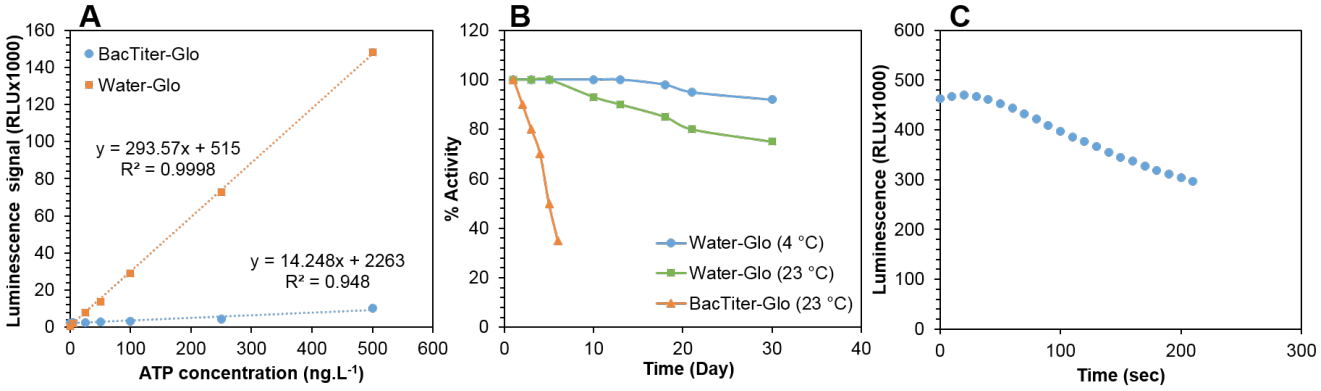

Fig. 2. (a) The measured luminescence signal of artificial seawater with different ATP standard concentrations ranging from 0 to 500 ng L^{-1} with ATP Water-Glo and BacTiter-Glo reagents. (b) The stability of ATP Water-Glo and BacTiter-Glo reagents over time at different storage temperatures. (c) Stability of luminescence signal over time for a seawater sample measured with ATP Water-Glo reagent.

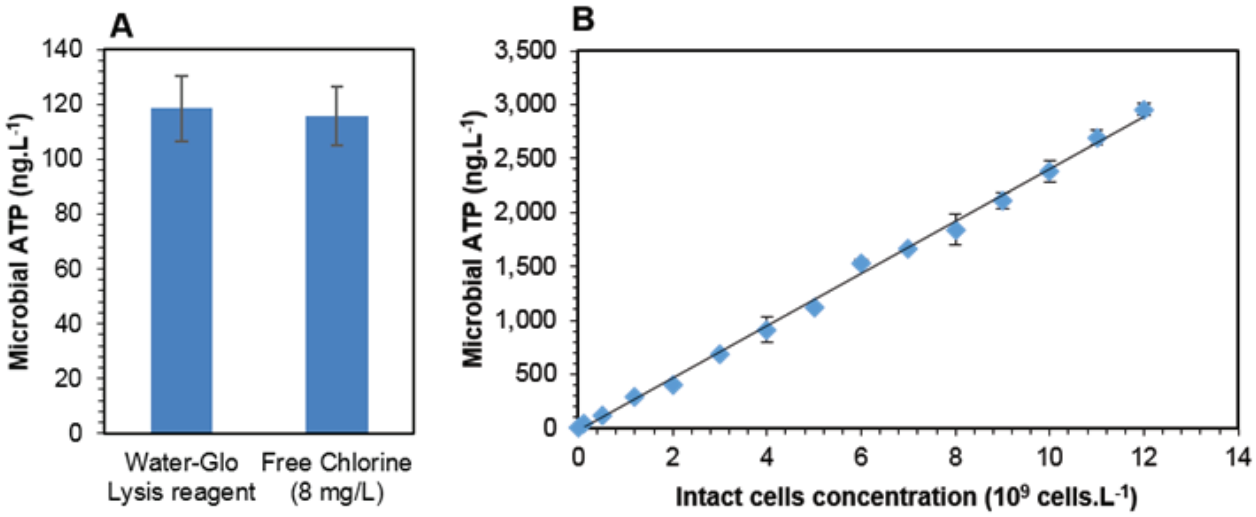

Fig. 3. (a) Comparing lysis efficiency of ATP Water-Glo and free chlorine in a raw seawater sample collected from Kamperland, the Netherlands. (b) Measured Microbial ATP and intact cell concentration (measured by FCM) in a concentrated seawater sample with 1.2×10^{10} cell L^{-1}. Marine microorganisms of 1 L seawater were concentrated in 30 mL.

influence on the measurement of bacterial growth potential as the sample is pasteurized and incubated in the dark.

The results of microbial cell concentrations in raw seawater that are effectively lysed by 100 μL of lysis reagent are presented in Fig. 3(b). A linear relationship was observed (4×10^3 cell mL^{-1} = 1.0 ng L^{-1}) between the intact cell concentration measured by FCM and the Microbial ATP concentration up to 1.2×10^{10} intact cell L^{-1}. This relation suggests that the use of 1:1 ratio of (seawater sample to Water-Glo lysis reagent) allows measurement of Microbial ATP up to 3,000 ng L^{-1}, which is equivalent to an AOC of 2,000 μg-glucose L^{-1}, based on a bacterial yield factor determined in our lab for North seawater (data not shown).

3.2.2. Water-Glo detection reagent

According to the manufacturer, the volume of Water-Go detection reagent should be equal to or greater than the (combined) volume of the seawater sample and lysis reagent. To demonstrate that sufficient Water-Glo detection reagent volume was used in this study, Microbial ATP concentration was measured by employing three different ratios of seawater sample volume:lysis reagent volume:detection reagent volume (100:100:100 μL, 100:100:200 μL and 100:200:300 μL). In the first ratio, half of the recommended volume (100 μL) of detection reagent (according to the manufacturer) was used whereas, in the second and third ratios, the recommended volume of detection reagent (200 and 300 μL) was tested. To convert the RLU signal into ATP concentration, calibration lines (Fig. 4(a)) for both free and total ATP were prepared for each test, considering the same total volume.

Microbial ATP concentration for the three different volumes of detection reagent (100, 200 and 300 μL) were similar (352 ± 6.2 ng L^{-1}) as shown in Fig. 4(b). From the obtained results for the 100:100:100 and 100:100:200 μL volume ratios (Fig. 4(b), first two columns), it appears that the recommended volume is sufficient to measure all Microbial ATP in the seawater samples. Moreover, the results of the 100:100:200 and 100:200:300 μL volume ratios (Fig. 4(b), last two columns) suggest that a high volume of detection reagent does not impact the final concentration. In conclusion, similar Microbial ATP concentrations with different volumes of Water-Glo detection reagent demonstrates that the use of 100–300 μL of detection reagent volume was sufficient to detect all of the Microbial ATP in the seawater samples.

3.3. Effect of pH and iron concentration on the luminescence signal

Since the ATP lysis and detection reagents are added directly to seawater, the chemical composition of the seawater sample may affect the measured ATP concentration. Several studies showed that pH, magnesium concentration and temperature play a role in the determination of ATP concentration [39–41].

In this research, the effect of seawater pH and iron concentration present in the sample has been investigated, since both acid- and iron-based coagulants are commonly applied in desalination plants.

High variations in the luminescence signal were observed at different seawater pH values in which the maximum luminescence signal was at seawater pH 8 – and the signal reduced by 40% and 60% at pH 7 and 8.5 (Fig. 5(a)), respectively. The variations may fluctuate depending on the buffering capacity of seawater. In SWRO plants, the pH of seawater is expected to decrease to less than 8 through the pre-treatment, depending on whether acid or coagulant is dosed and the respective concentration of each.

Similarly, the luminescence signal decreased (Fig. 5(b)) when the iron concentration in the seawater increased. For example, when the iron concentration increased from 0.1 to 10 mg-Fe(III) L^{-1}, the signal decreased by 62%. However, the iron concentration in the feedwater of the coagulation system of an SWRO plant is not expected to exceed 0.05 mg L^{-1} (maximum concentration recommended by the membranes supplier of DOW and Hydranautics). These results show that seawater pH and iron concentration affect the luminescence signal, and in turn ATP measurement.

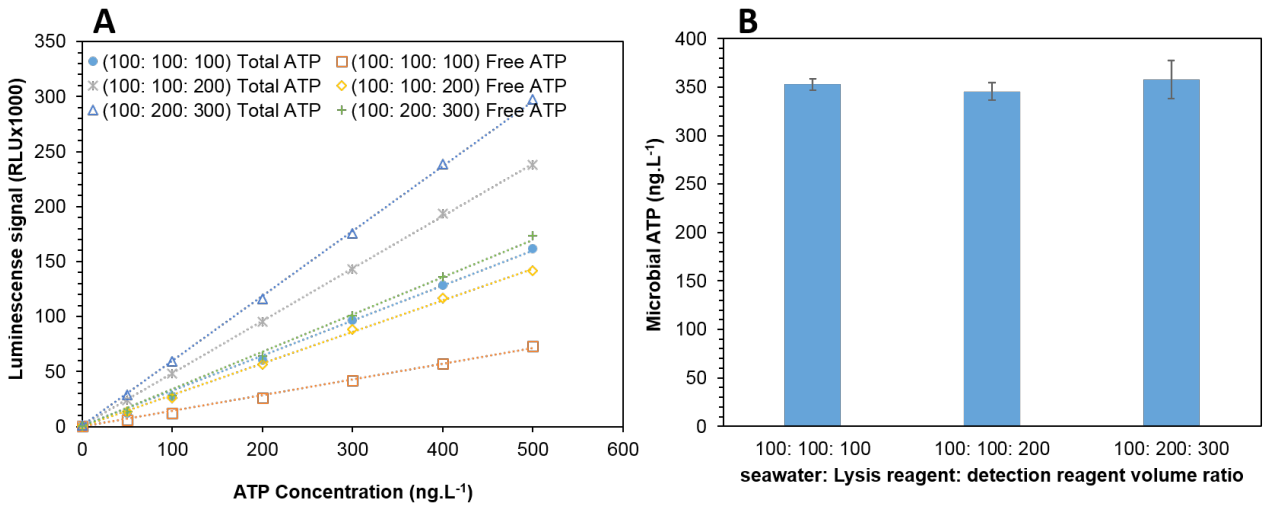

Fig. 4. (a) Calibration lines of the tested sample sets prepared by diluting ATP standard (100 nM ATP standard). (b) Measured Microbial ATP concentration of seawater with different volume ratio of ATP Water-Glo lysis reagent and ATP Water-Glo detection reagent.

In full-scale SWRO plants, seawater characteristics change through the pre-treatment train, which will affect the luminescence signal. For example, if the pH in the pre-treatment is decreased as a result of acid dosing, the measured luminescence signal of the seawater sample will be less than the actual signal – which in turn underestimates the measured Microbial ATP concentration. Therefore, to eliminate the luminescence signal variation caused by the differences in seawater characteristics, it is important to prepare different calibration lines taking these differences into consideration.

3.4. Calibration and limit of detection determination

3.4.1. Calibration line

Seawater characteristics (salinity, pH, etc.) play a significant role in the emitted luminescence signal as discussed earlier. Thus, to calculate ATP accurately, it is important to prepare a calibration curve with similar properties to the real seawater samples.

To investigate the optimum representative calibration line, the slope and intercept of different calibration lines were studied and compared with ATP standard addition to real seawater. These calibration lines were prepared with ASW, pasteurized seawater (70°C), sterilized seawater (121°C) and filtered seawater (0.1 μm). It was found that the slopes of all calibration lines were very similar (Table 2 and Fig. 6) ranging from 557 to 560 RLU ng^{-1}ATP L which demonstrates that all tested seawater samples (treated with filtration or autoclaving) have similar characteristics to real seawater (without any treatment). The high intercept (*y*-axis) values for real seawater, pasteurized seawater and filtered seawater calibration lines (65,365; 10,611 and 5,996 RLU, respectively) are due to Total/Free ATP concentration present in the sample. This result suggests that both sterilized seawater and ASW may be used to calibrate Microbial ATP in seawater since their slopes were similar to real seawater and their background levels are very low (intercept with *y*-axis; Fig. 6). However, preparing ASW with similar properties to real seawater is very tedious. Therefore, the use of sterilized seawater at 121°C is recommended.

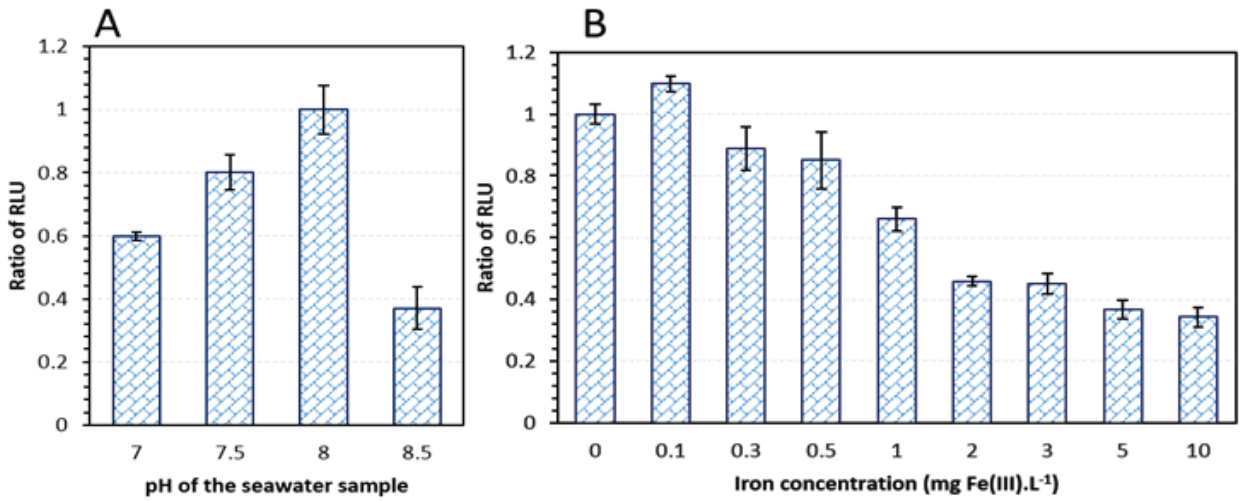

Fig. 5. The effect of (a) pH of the seawater sample and (b) iron concentration present in seawater on the luminescence signal.

Table 2
Calibration curves prepared in real seawater, pasteurized seawater, sterilized seawater, filtered (0.1 μm) seawater and artificial seawater

Calibration line properties		Real seawater – standard addition	Pasteurized seawater (70°C)	Sterilized seawater (121°C)	Filtered seawater (0.1 μm)	Artificial seawater
Calibration line	Slope of the calibration (RLU ng^{-1} ATP L)	559.9	559.5	556.7	557.9	558.4
	Regression coefficient (R^2)	0.998	0.992	0.999	0.996	0.999
Intercept point with *y*-axis	Average (RLU)	65,365	10,611	661	5,996	516
	Standard deviation (RLU)	742	82	20	249	23
	Variation coefficient (%)	1.2	1.5	4	4.2	4.5

Since it could be tedious to prepare several calibration lines as seawater characteristics may change along the pre-treatment processes. It is suggested to apply this method for monitoring of a sample over time, such as the determination of bacterial growth potential and AOC concentration and the monitoring of raw seawater. However, it can be applied for any seawater application as long as the calibration line represents the characteristics of the seawater sample (pH, iron concentration, etc.).

3.4.2. Limit of detection

The LOD was investigated for the recommended volume ratio (100 µL of seawater sample:100 µL of Water-Glo lysis reagent:200 µL of Water-Glo detection reagent). The LOD for Total ATP and Free ATP was 0.2 and 0.2 ng L^{-1}, respectively (Table 3) [38]. The combined LOD of Microbial ATP was 0.3 ng L^{-1}, which is approximately 1,200 cell mL^{-1} (using the correlation shown in Fig. 3(b)). The reported LOD of freshwater ATP methods is in the range between 0.05 and 5.1 ng L^{-1} [20,29,42,43].

As this method is intended for monitoring bacterial growth potential in SWRO plants, the method should be able to measure the lowest expected concentration in SWRO feedwater. However, there is no threshold concentration recommended for ATP in seawater in the literature. Thus, the threshold concentration for AOC in freshwater was used instead. Hijnen et al. [27] reported the lowest threshold concentration of AOC to avoid biofouling in freshwater RO system (1 µg-acetate L^{-1}), which is approximately 1×10^4 cell mL^{-1} [35]. The LOD of the direct ATP method in seawater (0.3 ng L^{-1}, 1,200 cell mL^{-1}) is approximately eight times lower than the reported threshold concentration (1×10^4 cell mL^{-1}) suggesting that ATP can be used to monitor bacterial growth potential in SWRO feedwater.

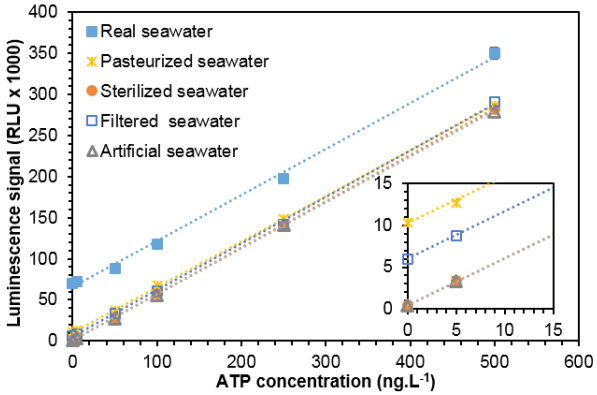

Fig. 6. Calibration lines prepared in real seawater, pasteurized seawater, sterilized seawater, filtered seawater and artificial seawater with standard addition of ATP ranging from 0 to 500 ng L^{-1}.

Table 3
The calculated limit of detection of Total ATP and Free ATP (n = 10)

	Total ATP	Free ATP
Average blanks (RLU)	584	191
Standard deviation (RLU)	16	11
LOD (ng L^{-1})	0.2 ± 0.1	0.2 ± 0.1
LOD of the method	0.3	

3.5. Application of the Microbial ATP method

3.5.1. Microbial ATP monitoring of raw seawater

The Microbial ATP concentration of seawater (the Netherlands) was regularly monitored (weekly to bi-weekly) over 2016. The concentration ranged from 25 to 1,037 ng L^{-1} with the lowest concentration observed during the winter months as shown in Fig. 7. It is not unlikely that microalgae were also lysed by the Water-Glo lysis reagent. In the winter, Microbial ATP ranged between 20 and 140 ng L^{-1} with an average of 52 ng L^{-1}. During the spring period, the Microbial ATP concentration started to increase above 100 ng L^{-1} and reached 1,000 ng L^{-1}. This increment could be due to the ATP extraction from the algal cells or due to the presence of sufficient nutrients released from algal cells during the bloom period which led to high bacterial growth. An algal bloom was noticed in April, when the algal cell counts increased from 10 to 1,000 cell mL^{-1}. Another possible reason would be due to the higher activity of microorganisms at higher temperatures. After the spring season, the Microbial ATP concentration declined to a range below 100 ng L^{-1}. The variation of marine Microbial ATP over time may indicate fluctuations in the amount of nutrients in seawater.

In the measured samples from the North Sea, the percentage of Microbial ATP ranged between 55% and 88% of the Total ATP with an average of 74% ± 9% (Fig. 8). Free ATP is not marginal and accounted (>12% of the Total ATP) which reveals that the use of Total ATP for indicating the microbial activity in the seawater instead of Microbial ATP may be misleading. Moreover, the abundance of Microbial ATP and Free ATP could vary depending on the seawater sample used and the bacterial growth phase.

3.5.2. Monitoring of Microbial ATP and bacterial growth in an SWRO desalination plant

The new reagents were used to measure the Microbial ATP concentration in a full-scale membrane-based desalination plant. Results showed the maximum Microbial ATP concentration (90 ng L^{-1}) in the raw seawater of Australian

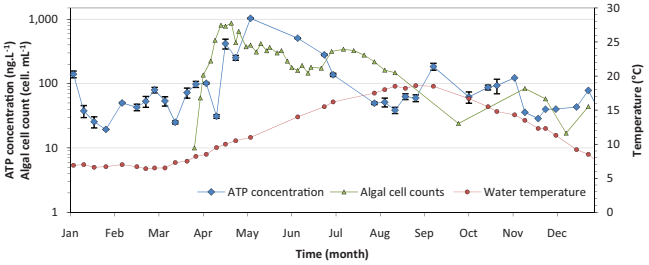

Fig. 7. Microbial ATP and algal cell concentrations in raw seawater collected from Kamperland (North Sea) between January and December 2016. All data points are plotted as average ± standard deviation (n = 3 each).

SWRO desalination plant (Fig. 9), which is relatively low compared with the measured Microbial ATP concentration in the North Sea during the spring (300–1,000 ng L^{-1}). The ATP concentration gradually decreased through the pre-treatment processes from 90 to 55, 38 and 19 ng L^{-1} after flocculation, DMF and after cartridge filtration, respectively. Different calibration lines were established for each sample due to the changes of the seawater matrix across the pre-treatment (pH, iron, magnesium, etc.), as presented earlier.

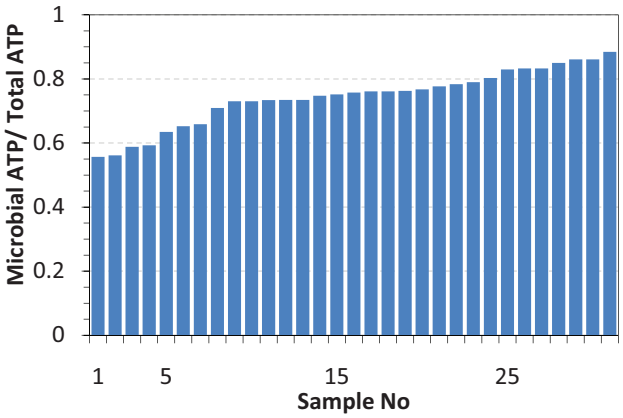

Fig. 8. The fraction of Microbial ATP over Total ATP of seawater from the North Sea (Kamperland, the Netherlands). All data bars are plotted as average (n = 3).

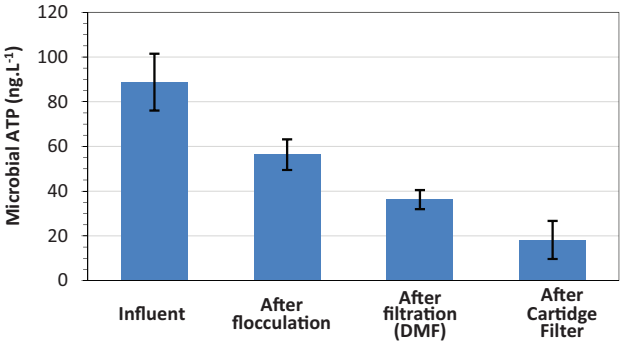

Fig. 9. Microbial ATP through the RO pre-treatment processes of an SWRO desalination plant in Australia (n = 3).

Microbial ATP measurement was also applied to monitor the bacterial growth potential across the pre-treatment processes of an SWRO plant (Fig. 10(a)). After bacterial inactivation of the seawater samples, the samples were inoculated with an average Microbial ATP concentration of 7.8 ± 1.7 ng L^{-1}. Bacteria started to grow immediately in seawater and reached a maximum growth within 2 d. Afterwards, Microbial ATP gradually decreased, either due to the partial decay of bacteria or because bacterial activity decreased due to the depletion of nutrients. As expected, the maximum bacterial growth was observed (305 ng L^{-1}) in raw seawater (Fig. 10(b)), indicating the highest potential of bacterial growth. Slightly lower potential of bacterial growth (262 ng L^{-1}) was noticed after coagulation and flocculation, while a significant reduction (>55%) of the bacterial growth potential was found after DMF – therefore, indicating a biologically active media filter. This high removal in the DMF coincided with the reported removal by Weinrich et al. [18] in which the removal in the sand filtration ranged between 25% and 70%. The maximum bacterial growth decreased modestly through the cartridge filter to 86 ng L^{-1}. This result shows that the seawater after pre-treatment still supports further bacterial growth as there are differences between the present Microbial ATP (19 ng L^{-1}) and the maximum Microbial ATP (86 ng L^{-1}) that can be found in the tested seawater. It should be noted that the protocol determination of bacterial growth potential based on Microbial ATP measurements using indigenous microbial culture will be discussed in depth in the following article.

The monitored Microbial ATP and bacterial growth potential based on Microbial ATP illustrate the applicability of the new developed reagents for measuring Microbial ATP and that this method can be applied to measure bacterial growth potential in seawater.

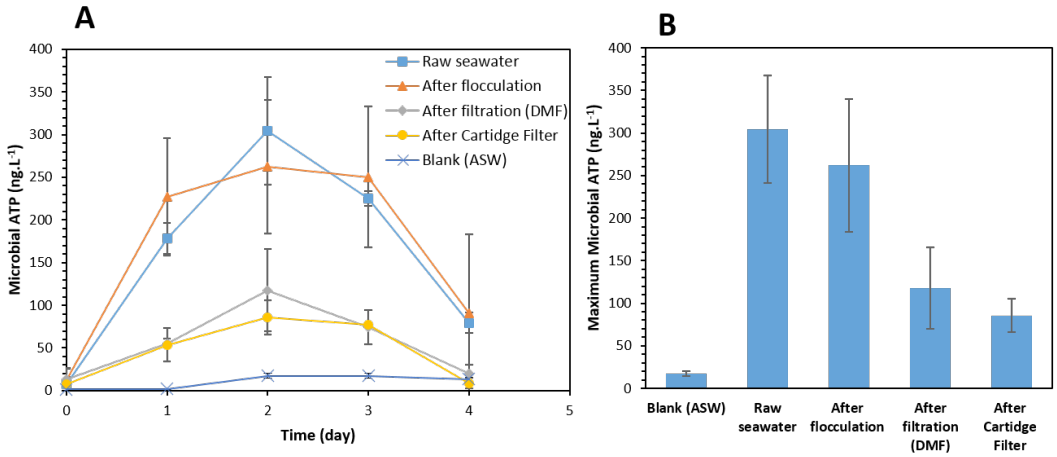

Fig. 10. (a) Bacterial growth over time and (b) maximum growth of different seawater samples collected through the pre-treatment processes of an SWRO desalination plant in Australia. Bacteria in the samples were inactivated and then inoculated with 7.8 ± 1.7 ng L^{-1} of Microbial ATP.

4. Conclusions

- The applicability of new reagents (Water-Glo lysis and detection reagent) to measure Microbial ATP directly in seawater has been demonstrated.
- Water-Glo lysis reagent shows strong lysis efficiency (similar to 8 mg L^{-1} free chlorine) in seawater.
- A linear relationship was observed between intact cell concentration measured by FCM and Microbial ATP concentration in seawater in the range 0–3,000 ng L^{-1} ATP (equivalent to 1.2×10^7 intact cell mL^{-1}).
- ATP Water-Glo detection reagent showed 20 times higher luminescence signal than the freshwater detection reagent, when used to measure ATP in seawater.
- To determine Microbial ATP directly in seawater, a calibration line with a similar water matrix to the actual seawater sample is required. Calibration is necessary as changes in pH and iron concentration affect the luminescence signal and the measured ATP concentration.
- The LOD of the direct method to determine Microbial ATP in seawater is 0.3 ng L^{-1} (equivalent to 1,200 cell mL^{-1}).
- Microbial ATP concentration in North Sea has been monitored and high seasonal variations were observed ranging from 20 to 1,000 ng L^{-1}.
- Microbial ATP has been applied to measure bacterial growth potential using an indigenous bacterial consortium in an SWRO desalination plant in Australia. A significant reduction (55%) in bacterial growth potential was noticed through dual media filtration with 4.5 mg-Fe(III) L^{-1} coagulant added prior to dual media filtration.
- Ongoing research will focus on the applicability of Microbial ATP for monitoring bacterial growth potential in SWRO plants around the world.

Acknowledgments

Special thanks go to David Grasso from Australia for access to the SWRO plant. This study was made possible by funding from the Promega Corporation (Madison, Wisconsin, USA).

Symbols

AOC	—	Assimilable organic carbon
ASW	—	Artificial seawater
ATP	—	Adenosine triphosphate
CFU	—	Colony forming units
CIP	—	Cleaning in place
DMF	—	Dual media filter
FCM	—	Flow cytometry
HPC	—	Heterotrophic plate count
LOD	—	Limit of detection
n	—	Number of samples
R^2	—	Regression coefficient
RLU	—	Relative light unit
RO	—	Reverse osmosis
SWRO	—	Seawater reverse osmosis
TDC	—	Total direct cell count
TDS	—	Total dissolved solids
TOC	—	Total organic carbon

References

[1] J.S. Vrouwenvelder, J.A.M. van Paassen, H.C. Folmer, J.A.M.H. Hofman, M.M. Nederlof, D. van der Kooij, Biofouling of membranes for drinking water production, Desalination, 118 (1998) 157–166.
[2] J.S. Vrouwenvelder, S.A. Manolarakis, J.P. van der Hoek, J.A.M. van Paassen, W.G.J. van der Meer, J.M.C. van Agtmaal, H.D.M. Prummel, J.C. Kruithof, M.C.M. van Loosdrecht, Quantitative biofouling diagnosis in full scale nanofiltration and reverse osmosis installations, Water Res., 42 (2008) 4856–4868.
[3] O. Ferrer, S. Casas, C. Galvañ, F. Lucena, A. Bosch, B. Galofré, J. Mesa, J. Jofre, X. Bernat, Direct ultrafiltration performance and membrane integrity monitoring by microbiological analysis, Water Res., 83 (2015) 121–131.
[4] S. Jeong, G. Naidu, S. Vigneswaran, C.H. Ma, S.A. Rice, A rapid bioluminescence-based test of assimilable organic carbon for seawater, Desalination, 317 (2013) 160–165.
[5] M.W. LeChevallier, Coliform regrowth in drinking water: a review, J. AWWA, 82 (1990) 74–86.
[6] D. van der Kooij, A. Visser, W.A.M. Hijnen, Determining the concentration of easily assimilable organic carbon in drinking water, J. AWWA, 74 (1982) 540–545.
[7] J.C. Joret, Y. Lévi, Méthode rapide d'évaluation du carbone éliminable des eaux par voies biologiques, La Tribune du CEBEDEAU, 510 (1986) 3–9.
[8] G. Stanfield, P.H. Jago, The Development and Use of a Method for Measuring the Concentration of Assimilable Organic Carbon in Water, WRC Environment Report, 1987.
[9] D. van der Kooij, H.R. Veenendaal, Regrowth Problems and Biological Stability Assessment in the Netherlands, D. van der Kooij, Paul W.J.J. van der Wielen, Eds. Microbial Growth in Drinking-Water Supplies: Problems, Causes, Control and Research Needs, IWA Publishing, 2014.
[10] L. Weinrich, M. LeChevallier, C.N. Haas, Contribution of assimilable organic carbon to biological fouling in seawater reverse osmosis membrane treatment, Water Res., 101 (2016) 203–213.
[11] I.C. Escobar, A.A. Randall, Sample storage impact on the assimilable organic carbon (AOC) bioassay, Water Res., 34 (2000) 1680–1686.
[12] L.A. Kaplan, T.L. Bott, D.J. Reasoner, Evaluation and simplification of the assimilable organic carbon nutrient bioassay for bacterial growth in drinking water, Appl. Environ. Microbiol., 59 (1993) 1532–1539.
[13] F.A. Kemmy, J.C. Fry, R.A. Breach, Development and operational implementation of a modified and simplified method for determination of assimilable organic carbon (AOC) in drinking water, Water Sci. Technol., 21 (1989) 155–159.
[14] F.A. Hammes, T. Egli, New method for assimilable organic carbon determination using flow-cytometric enumeration and a natural microbial consortium as inoculum, Environ. Sci. Technol., 39 (2005) 3289–3294.
[15] M.W. LeChevallier, N.E. Shaw, L.A. Kaplan, T.L. Bott, Development of a rapid assimilable organic carbon method for water, Appl. Environ. Microbiol., 59 (1993) 1526–1531.
[16] P.L. Haddix, N.J. Shaw, M.W. LeChevallier, Characterization of bioluminescent derivatives of assimilable organic carbon test bacteria, Appl. Environ. Microbiol., 70 (2004) 850–854.
[17] P.S. Ross, F. Hammes, M. Dignum, A. Magic-Knezev, B. Hambsch, L.C. Rietveld, A comparative study of three different assimilable organic carbon (AOC) methods: results of a round-robin test, Water Sci. Technol., 13 (2013) 1024–1033.
[18] L.A. Weinrich, O.D. Schneider, M.W. LeChevallier, Bioluminescence-based method for measuring assimilable organic carbon in pretreatment water for reverse osmosis membrane desalination, Appl. Environ. Microbiol., 77 (2011) 1148–1150.
[19] J.T. Staley, A. Konopka, Measurement of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats, Annu. Rev. Microbiol., 39 (1985) 321–346.
[20] G. Liu, E.J. Van der Mark, J.Q.J.C. Verberk, J.C. Van Dijk, Flow cytometry total cell counts: a field study assessing microbiological water quality and growth in unchlorinated drinking water distribution systems, Biomed Res. Int., 2013 (2013) 10.

[21] J.R. Postgate, Chapter XVIII viable counts and viability, Methods Microbiol., 1 (1969) 611–628.
[22] M. Montes, E.A. Jaensson, A.F. Orozco, D.E. Lewis, D.B. Corry, A general method for bead-enhanced quantitation by flow cytometry, J. Immunol. Methods, 317 (2006) 45–55.
[23] A.W. Thompson, G. van den Engh, A multi-laser flow cytometry method to measure single cell and population-level relative fluorescence action spectra for the targeted study and isolation of phytoplankton in complex assemblages, Limnol. Oceanogr. Methods, 14 (2016) 39–49.
[24] J.R. Knowles, Enzyme-catalyzed phosphoryl transfer reactions, Annu. Rev. Biochem., 49 (1980) 877–919.
[25] J.J. Webster, G.J. Hampton, J.T. Wilson, W.C. Ghiorse, F.R. Leach, Determination of microbial cell numbers in subsurface samples, Ground Water, 23 (1985) 17–25.
[26] D.M. Karl, Cellular nucleotide measurements and applications in microbial ecology, Microbiol. Rev., 44 (1980) 739–796.
[27] W.A.M. Hijnen, D. Biraud, E.R. Cornelissen, D. van der Kooij, Threshold concentration of easily assimilable organic carbon in feedwater for biofouling of spiral-wound membranes, Environ. Sci. Technol., 43 (2009) 4890–4895.
[28] O. Holm-Hansen, C.R. Booth, The measurement of adenosine triphosphate in the ocean and its ecological significance, Limnol. Oceanogr., 11 (1966) 510–519.
[29] F. Hammes, F. Goldschmidt, M. Vital, Y. Wang, T. Egli, Measurement and interpretation of microbial adenosine tri-phosphate (ATP) in aquatic environments, Water Res., 44 (2010) 3915–3923.
[30] A. Magic-Knezev, D. van der Kooij, Optimisation and significance of ATP analysis for measuring active biomass in granular activated carbon filters used in water treatment, Water Res., 38 (2004) 3971–3979.
[31] J.M. Veza, M. Ortiz, J.J. Sadhwani, J.E. Gonzalez, F.J. Santana, Measurement of biofouling in seawater: some practical tests, Desalination, 220 (2008) 326–334.
[32] G.L. Amy, S.G. Salinas Rodriguez, M.D. Kennedy, J.C. Schippers, S. Rapenne, P.J. Remize, C. Barbe, C.L. de O. Manes, N.J. West, P.L. Lebaron, D. van der Kooij, H. Veenendaal, G. Schaule, K. Petrowski, S. Huber, L.N. Sim, Y. Ye, V. Chen, A.G. Fane, Water Quality Assessment Tools, Membrane-based Desalination: An Integrated Approach (MEDINA), IWA Publishing, 2011, pp. 3–32.
[33] C. van Slooten, T. Wijers, A.G.J. Buma, L. Peperzak, Development and testing of a rapid, sensitive ATP assay to detect living organisms in ballast water, J. Appl. Phycol., 27 (2015) 2299–2312.
[34] F.X. Simon, E. Rudé, J. Llorens, S. Baig, Study on the removal of biodegradable NOM from seawater using biofiltration, Desalination, 316 (2013) 8–16.
[35] F. Hammes, C. Berger, O. Köster, T. Egli, Assessing biological stability of drinking water without disinfectant residuals in a full-scale water supply system, J. Water Supply Res. Technol. AQUA, 59 (2010) 31–40.
[36] L.O. Villacorte, Algal Blooms and Membrane Based Desalination Technology, Delft University of Technology, CRC Press/Balkema, PO Box 11320, 2301 EH Leiden, The Netherland, 2014.
[37] A. Nescerecka, T. Juhna, F. Hammes, Behavior and stability of adenosine triphosphate (ATP) during chlorine disinfection, Water Res., 101 (2016) 490–497.
[38] I. Taverniers, M. De Loose, E. Van Bockstaele, Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance, TrAC, Trends Anal. Chem., 23 (2004) 535–552.
[39] M.J. Hubley, B.R. Locke, T.S. Moerland, The effects of temperature, pH, and magnesium on the diffusion coefficient of ATP in solutions of physiological ionic strength, Biochim. Biophys. Acta, 1291 (1996) 115–121.
[40] C. Bergman, Y. Kashiwaya, R.L. Veech, The effect of pH and free Mg^{2+} on ATP linked enzymes and the calculation of Gibbs free energy of ATP hydrolysis, J. Phys. Chem. B, 114 (2010) 16137–16146.
[41] J. Ma, A.M. Ibekwe, M. Leddy, C.-H. Yang, D.E. Crowley, Assimilable organic carbon (AOC) in soil water extracts using vibrio harveyi BB721 and its implication for microbial biomass, PLoS One, 7 (2012) e28519.
[42] Ó.K. Vang, C.B. Corfitzen, C. Smith, H.-J. Albrechtsen, Evaluation of ATP measurements to detect microbial ingress by wastewater and surface water in drinking water, Water Res., 64 (2014) 309–320.
[43] S. Velten, F. Hammes, M. Boller, T. Egli, Rapid and direct estimation of active biomass on granular activated carbon through adenosine tri-phosphate (ATP) determination, Water Res., 41 (2007) 1973–1983.