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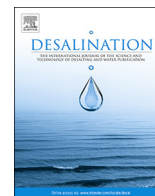
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## ATP measurement in seawater reverse osmosis systems: Eliminating seawater matrix effects using a filtration-based method



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### ABSTRACT

A direct method for measuring adenosine-triphosphate (ATP) in seawater was developed recently, in which commercial reagents are added directly to seawater. However, calibration is required if seawater quality changes (such as changes in salinity, pH,  $Mg^{2+}$ ,  $Fe^{3+}$ ) as the seawater matrix interferes with ATP measurement. In this research, a 0.1  $\mu m$  filtration process is introduced to eliminate such interferences. In addition, a filter rinsing step with sterilized artificial seawater is proposed to eliminate interference of free ATP.

The ATP-filtration method is fast ( $< 5$  min), reproducible ( $VC = 7\%$ ), six times more sensitive than the direct ATP-method and correlates ( $R^2 = 0.72$ ,  $n = 100$ ) with intact cell concentration. Microbial ATP concentration measured using the ATP-filtration method and the ATP-direct method were comparable. Microbial ATP measured along the treatment train of a full-scale seawater reverse osmosis (SWRO) plant decreased from 530 in the raw seawater to 10 ng-ATP/L after pre-treatment and to 0.5 ng-ATP/L in the SWRO permeate. The method was also applied to monitor bacterial growth potential (BGP) across the pre-treatment train of a (pilot) seawater desalination plant, where the removal of BGP through the media filtration and ultrafiltration was 44% and 7%, respectively.

### 1. Introduction

In reverse osmosis (RO) desalination, microbial quantification has been implemented: (i) to quantify biomass accumulation on RO membranes for biofouling diagnostics [1,2], (ii) to measure biomass in the feed water and across pre-treatment trains in RO plants [3,4], (iii) as a biomass parameter for the determination of nutrients (carbon) [5–7], and (iv) to measure bacterial growth potential [6,8,9].

The common methodologies to quantify microbes are heterotrophic plate counts (HPC's) and total direct counts (TDC's). HPC has been used

to monitor microbial populations in seawater [10], drinking water treatment [11] and distribution systems [12] but it is a laborious and time consuming method. HPC is also limited to the enumeration of cultivable bacteria, which may comprise  $< 1\%$  of the active bacterial population in natural water [13,14]. TDC enumerates the total numbers of cells but does not distinguish between active and inactive cells and is limited to samples that have high cell concentrations ( $> 10^7$  cells/mL) [15]. To avoid these limitations, flow cytometry (FCM), and adenosine triphosphate (ATP) have been proposed as alternative methods. FCM offers rapid enumeration of the total number of bacterial cells in water

**Abbreviations:** ANOVA, analysis of variation; AOC, assimilable organic carbon; ASTM, American Society for Testing and Materials; ASW, artificial seawater; ATP, adenosine-triphosphate; VC, variation coefficient; DMF, dual media filter; DMSO, dimethyl sulfoxide; FCM, flow cytometry; HPC, heterotrophic plate count; ICC-FCM, intact cell concentration measured by flow cytometry; LOD, limit of detection; n, number of samples; UV, ultraviolet;  $R^2$ , regression coefficient; Rho, Spearman regression; RLU, relative light unit; RO, reverse osmosis; SWRO, seawater reverse osmosis; TDC, total direct bacterial cell count; TDS, total dissolved salts; TOC, total organic carbon

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using bacterial DNA staining. ATP is the energy source in living organisms and it is used as a measure for the amount of active biomass [16–18]. ATP and FCM have attracted increasing interest because such methods are considered to be more accurate, rapid, and quantitative, can detect both cultivable and non-cultivable microorganisms, can be automated and are easy to perform [19,20].

In freshwater aquatic environments, ATP has been used to monitor water quality across treatment trains in drinking water plants [21–23], measuring active biomass on granular activated carbon, sand and anthracite grains [24], biostability of drinking water and biofilm formation in distribution systems [25–27] and determining ATP in ground water [28,29]. ATP in aquatic systems can be found within live/active microorganisms (microbial ATP) or ATP in the water, which has been released from dead or stressed living microorganisms (free ATP). Various studies have been conducted to evaluate microbial activity in freshwater either using total ATP (microbial ATP and free ATP) or only microbial ATP [2,24,30]. However, the application of ATP in seawater is limited due to the interference of salts in the luciferase-luciferin reaction. The high concentration of salt in seawater has been demonstrated by van der Kooij and Veendaaal [31] to cause substantial reduction of the emitted light during the enzymatic ATP luciferase-luciferin reaction at a high salt concentration ( $> 10$  g/L).

The standard method for microbial ATP determination in all types of water used by the American Society for Testing and Materials (ASTM) [32] includes a filtration process (0.45  $\mu$ m pore size) in which the filter is transferred and placed in tris-buffer after filtration. The filter and the tris-buffer are heated at 100 °C to extract the microbial ATP from microorganisms. This method is complicated, and contamination may be introduced while performing the measurement. Van Slooten et al. [33] followed the same concept outlined by ASTM to quantify microbial ATP concentrations of living organisms (with a size between 10 and 50  $\mu$ m) in ballast water using a 10  $\mu$ m pore sized nylon filter. Marine microorganisms captured on the filter were flushed out with Milli-Q water to eliminate interference from salt, and then the filter, including the retained microorganisms, was placed into a cuvette with 2 mL of Milli-Q water before analysing microbial ATP using freshwater reagents. This method is also complicated, and the use of Milli-Q water to flush marine microorganisms can rapidly burst bacterial cells due to osmotic shock resulting in loss of microbial ATP.

Recently, direct quantification of microbial, free, and total ATP (total ATP is the sum of microbial ATP and free ATP) determination in seawater was proposed by Abushaban et al. [34] using new commercial reagents (Water-Glo kit, Promega, USA). The new reagents overcome the luciferin-luciferase problem and provide a high luminescence signal even in the presence of salt. The reagents are added directly to seawater for microbial ATP extraction and detection. The method is simple, direct, allows ATP determination at a low concentration level (limit of detection (LOD) = 0.3 ng-ATP/L) and is promising for monitoring microbial growth potential in SWRO systems. Nevertheless, a calibration line is needed each time the seawater quality changes (as any changes in pH, total dissolved salt (TDS),  $Mg^{2+}$ ,  $Fe^{3+}$ , turbidity) affect ATP measurement. Preparing several calibration lines may be tedious in some applications, such as along the pre-treatment train of SWRO plants since pH,  $Fe^{3+}$ , turbidity, etc. will vary depending on the applied settings in each pre-treatment step.

In this study, the interference of salt in microbial ATP determination was eliminated using a filtration step. Filtration allows the capture of marine microorganisms on a filter surface. Thus, eliminating the seawater matrix effect. To remove free ATP present in the filter holder after filtration, the captured microorganisms on the filter surface are rinsed with sterilized artificial seawater. Moreover, the use of filtration can improve the detection limit of the method by increasing the sample volume, enabling measurement of samples that have low ATP concentrations (such as after microfiltration and ultrafiltration). The following aspects have been addressed in this work:

- Pore size of the filter and flushing of free ATP from the filter holder.
- Method properties, including the limit of detection, reproducibility, and the correlation with intact cell counts measured by FCM in North Sea water.
- Comparing the ATP-direct method and the new ATP-filtration method.
- Monitoring bacterial growth potential along the pre-treatment line of a seawater pilot plant.
- Measuring microbial ATP concentration across the pre-treatment train of a full-scale SWRO desalination plant.

## 2. Materials and methods

### 2.1. Sample collection and storage

Natural coastal seawater samples from the North Sea water (Kamperland, The Netherlands) were collected from September 2017 to May 2018 in sterile 500 mL glass sampling bottles with tight-fitting screw caps. The samples were transported (110 km) for analysis to IHE Delft (Delft, The Netherlands) in a cooler (5 °C). The characteristics of the collected seawater samples are: pH = 7.9  $\pm$  0.1, TDS = 32.5  $\pm$  0.8 g/L, electrical conductivity = 52.6  $\pm$  1.2 mS/cm, TOC = 1.28  $\pm$  0.85 mg/L, UV<sub>254</sub> = 0.045  $\pm$  0.009 1/cm, total bacterial count measured with FCM = 0.9  $\pm$  0.28  $\times 10^6$  cells/mL.

### 2.2. Preparation of ASW

Artificial seawater containing all ions (ASW<sub>all ions</sub>) naturally present in seawater was prepared using Milli-Q water (Milli-Q® water Optimized purification, 18.2 M $\Omega$ -cm at 25 °C, Millipore, USA) and analytical or reagent-grade inorganic salts (Merck, USA) with ion concentrations similar to the average global seawater [35] (23.668 g/L NaCl, 10.873 g/L MgCl<sub>2</sub>·6H<sub>2</sub>O, 3.993 g/L Na<sub>2</sub>SO<sub>4</sub>, 1.54 g/L CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.739 g/L KCl, 0.213 g/L NaHCO<sub>3</sub>, and 0.002 g/L Na<sub>2</sub>CO<sub>3</sub>). Similarly, ASW<sub>NaOH + NaHCO<sub>3</sub></sub> was prepared using 33.2 g/L NaCl and 0.213 g/L NaHCO<sub>3</sub>. All salts except sodium bicarbonate were mixed (150 rpm) with Milli-Q water for 24 h and autoclaved at 100 °C for 20 min. Sodium bicarbonate was added after autoclaving because its melting point is 50 °C.

### 2.3. Microbial ATP measurement

Fig. 1 presents the protocol employed in the ATP-filtration method. Seawater samples (5 mL) were filtered through disposable sterile 0.1  $\mu$ m PVDF membrane filters (Millex GP, Merck Millipore, USA). Two millilitres of sterilized ASW<sub>all ions</sub> was filtered through the same filter to flush out the remaining volume of seawater in the filter holder ensuring complete removal of free ATP. Afterwards, 5 mL of the Water-Glo lysis reagent (Promega Corp., USA) was added to extract microbial ATP from the captured microorganisms, and then the filtrate was collected in 15 mL sterile centrifuge tubes. The filtrate and the ATP Water-Glo detection reagent were separately and simultaneously heated to 38 °C in a dry heating block (AccuBlock™ Digital, Labnet, USA). A 100  $\mu$ L aliquot of the filtrate was added to 100  $\mu$ L of the ATP Water-Glo detection reagent, and then the luminescence generated was measured with a GloMax®-20/20 instrument (Promega Corp.). No effect of the filtration rate (range: 50 to 400 L/m/h) could be found on the measured microbial ATP concentration.

The measured emitted light in relative light units (RLU) was converted to microbial ATP concentration based on a calibration line. To prepare a calibration line, the procedure described above was followed but without seawater sample filtration. The filtrate of the Water-Glo lysis reagent and standard ATP (1000 ng-ATP/L, Promega Corp., USA) were used to prepare standard ATP solutions ranging between 0 and 500 ng-ATP/L.

To compare the measured microbial ATP using the ATP-filtration

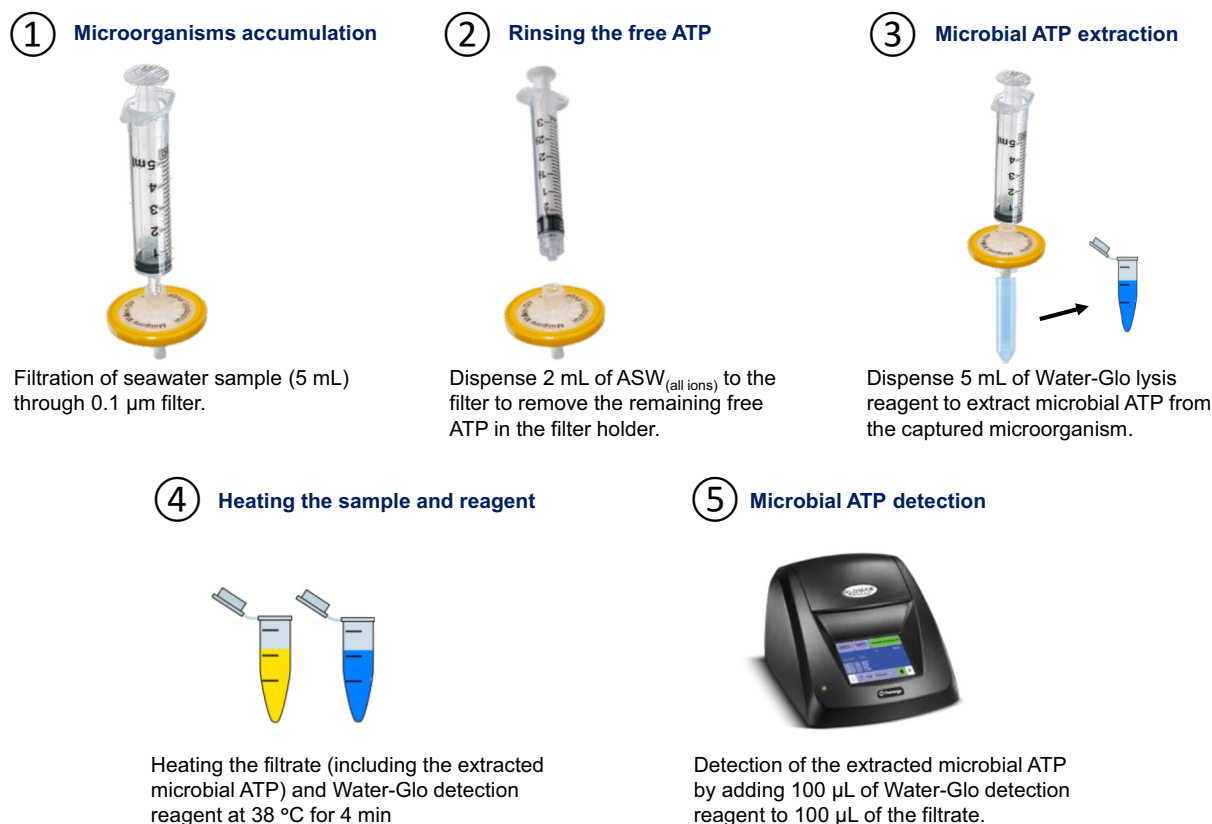


Fig. 1. Protocol of the ATP-filtration method to measure microbial ATP in (sea) water.

method with the measured microbial ATP using the ATP-direct method, 125 seawater samples from North Sea water and Tasman seawater were tested using both protocols. The procedure of the ATP-direct method in seawater, described in Abushaban et al. [34], was followed. The following diagram (Fig. 1) depicts the protocol that has been followed to monitor microbial ATP concentration using the ATP-filtration method.

#### 2.4. Tested variables in the ATP-filtration method

Several variables that might affect the performance of the ATP-filtration method were tested, including the filter pore size, rinsing free ATP from the filter holder, and the effect of the seawater sample volume.

There is evidence that a significant amount of marine bacteria could pass through membrane filters with a pore size of 0.45 µm and even through 0.2 µm [36,37]. A comparison was made between 0.1 µm PVDF sterilized filters, 0.22 µm PES sterilized filters and 0.45 µm PVDF sterilized filters (Millex GP, Merck Millipore, USA) based on retained microbial ATP on the filter surface (measured using the ATP-filtration method). The properties of the filters tested are shown in Table 1.

To test the effect of rinsing the filter on the removal of free ATP from the holder, different options were tested, including: (1) no rinsing, (2) rinsing with 2 mL of demineralized water, (3) rinsing with 2 mL of ASW comprising sodium, chloride, and bicarbonate ions (ASW<sub>NaCl + NaHCO<sub>3</sub></sub>), and (4) rinsing with 2 mL of ASW containing all the major ions in seawater (ASW<sub>all ions</sub>).

The effect of the seawater sample volume was also studied by measuring the luminescence signal and the calculated microbial ATP of different sample volumes ranging between 1 and 30 mL for seawater samples.

Table 1

Properties of the tested membrane filters [38].

Pore size	Millex-VV syringe filter, 0.1 µm	Millex-GP syringe filter, 0.22 µm	Millex-HV syringe filter, 0.45 µm
Sterility	Sterile	Sterile	Sterile
Material	Polyvinylidene fluoride (PVDF)	Polyethersulfone (PES)	Polyvinylidene fluoride (PVDF)
Wettability	Hydrophilic	Hydrophilic	Hydrophilic
Maximum inlet pressure	10 bar	10 bar	10 bar
Bubble point	73 psi (5.1 bar)	57 psi (3.9 bar)	25 psi (1.7 bar)
Filter diameter	33 mm	33 mm	33 mm
Filtration area	4.5 cm <sup>2</sup>	4.5 cm <sup>2</sup>	4.5 cm <sup>2</sup>

#### 2.5. Total intact cell counts using flow cytometry (ICC-FCM)

Intact cells were counted by double DNA staining and flow cytometric analysis as described elsewhere [39]. In brief, 10 µL of the SYBR green solution was mixed with 10 µL of propidium iodide (1 mg/mL) and 980 µL of 0.22 µm filtered DMSO solution. SYBR green is a cell-permeable DNA binding dye and can bind to DNA of either intact or damaged cells, while propidium iodide is a membrane-impermeable DNA binding dye and binds to DNA in cells that have only lost their membrane integrity. Simultaneous staining with SYBR green and propidium iodide allows a distinction to be made between intact and damaged bacteria. The water sample (500 µL) was first heated to 36 °C for 5 min and then stained by adding 5 µL of the SG/PI solution. The stained sample was then incubated at 36 °C for 10 min. Five hundred microlitres of the stained sample was injected at medium speed into the flow cytometer (BD Accuri C6). The result was visualized in a special gate designed for seawater samples and counted using a two-dimensional FL1-A (emission filter 533/30) vs. a FL3-A (emission filter 670 LP) log-scale density plot. The range of bacterial counts can be as low as

100 cells/mL and as high as  $10^7$  cells/mL.

### 2.6. Comparing the ATP-filtration and ATP-direct methods in seawater applications

The ATP-filtration method was compared to the ATP-direct method. The comparison was made based on the microbial ATP concentration in 2 different applications:

- (1) In a full-scale desalination plant, seawater samples are collected along the treatment train of an SWRO desalination plant. The treatment line of the plant included a drum screen, coagulation, dual media filtration, cartridge filter, 2 pass RO membrane, and remineralization.
- (2) Several locations: 125 raw seawater samples were collected from the Tasman Sea (Australia) and the North Sea (The Netherlands).

### 2.7. Monitoring of bacterial growth potential in a seawater pilot plant

Seawater samples were collected from raw seawater after a media filter (AFM® Active Filter Media, Grade 2, RD397) and after ultra-filtration from the pre-treatment of pilot-scale desalination plant (Kamperland, The Netherlands). The samples were pasteurized at 70 °C for 1 h and inoculated with 10,000 intact cells/mL (measured by flow cytometry) using an indigenous microbial inoculum from the same seawater source. The samples were incubated at 30 °C, and microbial ATP concentrations were monitored daily using the ATP-filtration method to assess the bacterial growth potential.

### 2.8. Statistical analysis

The average of triplicate measurements was reported, and the standard deviation was shown as the positive and negative error bars. The linear regression and Spearman correlation were calculated to assess the correlation between microbial ATP and intact cell concentration and the correlation between the ATP-filtration and ATP-direct methods. Moreover, an analysis of variation (ANOVA) was used to determine the significance of the correlation, including a P-value test and *t*-test of 2 samples assuming equal variances.

## 3. Results and discussion

### 3.1. Selection of filter pore size

Filter pore size may play a significant role in the determination of microbial ATP of each method. As mentioned earlier, ASTM method (ASTM-D4012) uses a 0.45 µm filter to quantify microbial ATP in water. However, Bowman et al. [40] reported that smaller organisms were not retained by a 0.45 µm filter [41], and Macdonell and Hood [37] observed that smaller marine bacteria (*Bdellovibrio*) in the Gulf of Mexico can even pass through 0.2 µm filters. To verify whether small size bacteria significantly influence the measured microbial ATP, a comparison of three filter pore sizes (0.1 µm, 0.22 µm and 0.45 µm) was performed for North Sea water. Higher microbial ATP concentrations were found using a smaller filter pore size for all seawater samples measured over 8 months, which ranged between 265 and 1335 ng-ATP/L (Fig. 2). The microbial ATP measured using a 0.22 µm filter was 12 to 47% lower than using a 0.1 µm filter. Furthermore, the microbial ATP measured using a 0.45 µm filter was 16 to 50% lower than the microbial ATP measured using a 0.1 µm filter. The variation in these percentages could be due to seasonal variations of microbial ATP or the prevalence of certain seasonal bacterial species smaller than 0.22 µm. At the beginning of spring (March and April), a high microbial ATP concentration was captured on 0.1 µm filter compared to the captured microbial ATP concentration on the 0.22 and 0.45 µm filter, which could be due to the algal bloom and the presence of microalgae.

The retention of microorganisms on a 0.1 µm filter was tested by measuring intact cell concentration in the raw seawater and the filtrate. In average, > 99.9% of microorganisms were retained on the 0.1 µm filter (see Table S1). Accordingly, the 0.1 µm filter was selected for the filtration process. This conclusion is in agreement with the findings of Wang et al. [42] and Hammes et al. [22] in which freshwater bacteria were detected in the filtrate of the 0.22 µm filter and thus used the 0.1 µm filter to distinguish between microbial ATP and free ATP in freshwater. This conclusion was drawn without testing the effect of membrane materials, which needs further study.

### 3.2. Removal of free ATP

After the seawater sample filtration, a small volume (0.45 mL, < 10% of the sample) of the seawater remains in the filter holder. The remaining volume also includes free ATP (< 5% of the total concentration, assuming free ATP is 50% of total ATP) which interferes with the measured microbial ATP concentration. The interference of free ATP is variable depending on the free ATP concentration in the sample. This retained volume can be removed either by air flushing or rinsing (e.g., with demineralized water). The use of air flushing is impractical with a 0.1 µm filter at a lab scale because the bubble point of a 0.1 µm filter is approximately 5 bar [38]. This might be ameliorated by using a pump operating at a constant flow to overcome the bubble point.

To assess the effectiveness of removing free ATP using rinsing, the microbial ATP of the seawater sample was measured and compared using four different rinsing conditions: (1) No rinsing, (2) rinsing with demineralized water, (3) rinsing with ASW comprising sodium, chloride and bicarbonate ions ( $ASW_{NaCl+NaHCO_3}$ ), and (4) rinsing with ASW containing all major ions in seawater ( $ASW_{all\ ions}$ ).

In the absence of rinsing, microbial ATP was similar to microbial ATP measured with  $ASW_{all\ ions}$  (Fig. 3) due to the fact that the free ATP concentration in the tested sample was insignificant. Although eliminating the rinsing option appears to be the most obvious solution, the variability of free ATP interference makes it less preferable since it depends on the free ATP concentration present in the seawater sample which could be very significant in some samples. One may suggest that this interference is taken into consideration by calibration. Indeed, this is theoretically applicable; however, a calibration line is then required for each sample, which is also impractical. On the other hand, the use of demineralized water to flush seawater (including free ATP in the filter holder) showed a negative effect on the microbial ATP measurement (Fig. 3) because the measured concentration was 66% lower than the microbial ATP concentration measured with other rinsing options. Rinsing marine microorganisms with demineralized water can rapidly burst the captured microorganisms on the filter surface due to osmotic shock, which results in a significant loss of microbial ATP. The survival of marine microorganisms rinsed with different salt concentrations ranging between 0 and 60 g/L was tested, which showed that marine microorganisms (North Seawater) can survive when rinsed with an artificial solution with a salt concentration ranging between 10 and 35 g/L (Fig. S1).

The microbial ATP concentration using  $ASW_{(NaCl+NaHCO_3)}$  was 10% less than the microbial ATP concentration measured using  $ASW_{all\ ions}$  (Fig. 3). This difference could be due to the absence of some ions in the  $ASW_{(NaCl+NaHCO_3)}$  such as calcium and magnesium [43,44]. It was found that calcium and magnesium are essential to the survival of marine microorganisms with regards to cell viability, as shown in the Supplementary data Figs. S2 and S3. It should be noted that the use of ASW comprising all ions overcomes the interference of free ATP because  $ASW_{all\ ions}$  is sterile and has constant properties. Consequently, a rinsing step using  $ASW_{(all\ ions)}$  was used to flush seawater.



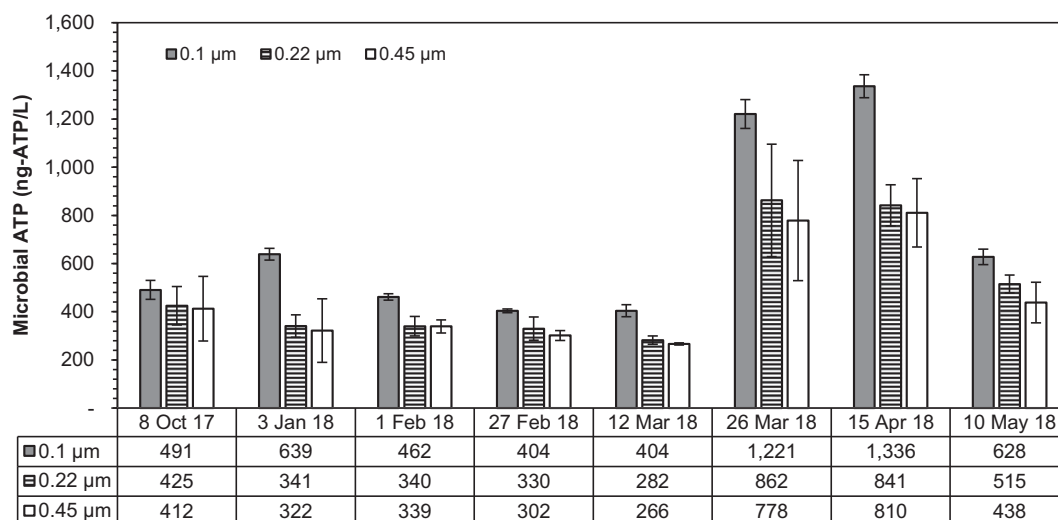


Fig. 2. Microbial ATP concentration measured using the ATP-filtration method with 0.1 μm, 0.22 μm and 0.45 μm filter. Seawater samples were collected from the North Sea.

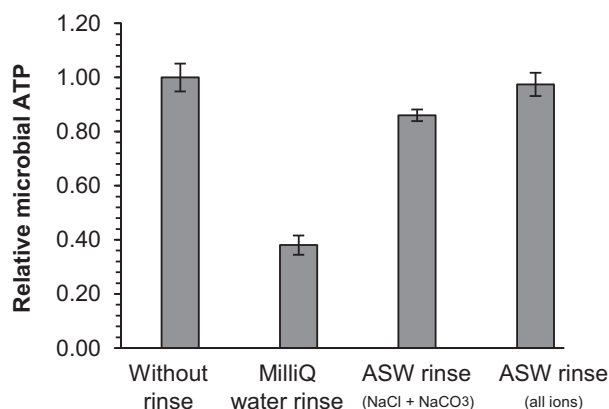


Fig. 3. Tested rinsing solutions to remove/flush the remaining seawater from the filter holder (including free ATP). Two mL of each solution was applied to remove the free ATP.

### 3.3. Limit of detection and sample volume

The LOD of the ATP-filtration method was calculated based on the average of 10 blanks plus 3 times the standard deviation of the 10 blanks (95% confidence level). ASW<sub>all ions</sub> was used as a blank sample for LOD determination. The measured LOD of the ATP-filtration method was 0.06 ng-ATP/L based on the proposed volume ratio (1:1, seawater sample: Water-Glo lysis reagent). Abushaban et al. [34] showed that the use of a 1:1 ratio of the seawater sample to lysis reagent allows measurement of microbial ATP up to 3000 ng-ATP/L, which was equivalent to about  $1.2 \times 10^{10}$  cells/L (measured by FCM). Additionally, to measure samples below the LOD, a higher volume of seawater can be filtered. For example, to measure a sample with 0.006 ng-ATP/L, the filtered seawater volume needs to be > 10 times (> 50 mL) that of the lysis reagent volume. By controlling the sample volume, the ATP-filtration method can be more sensitive than the reported LOD of the ATP-direct method (LOD = 0.3 ng-ATP/L) [34].

The higher sensitivity of the ATP-filtration method is due not only to the sample volume but also to the high measured signal using the Water-Glo reagent because the interference of the seawater salts with the reagent was eliminated. The difference between the luminescence signal of the ATP-direct method and the luminescence signal of the ATP-filtration method can be seen in Fig. 4, in which the calibration lines of both methods were compared. The slope of the ATP-filtration

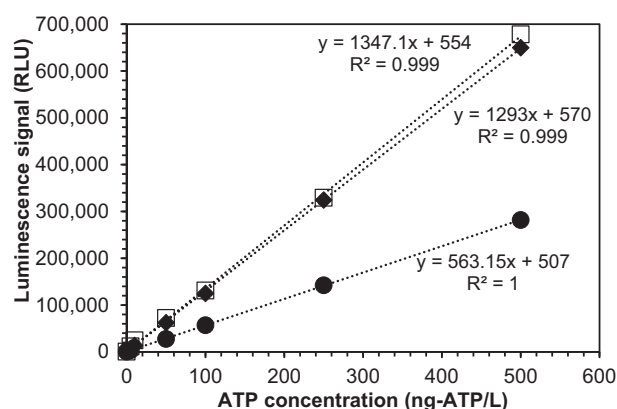


Fig. 4. Comparison between the calibration curves of the ATP-direct method and the ATP-filtration method in freshwater and seawater. Symbols: (●) ATP-direct method calibration line in seawater, (◆) ATP-filtration method calibration line in seawater, and (□) ATP-filtration method calibration line in freshwater.

method calibration line (1293 RLU·L/ng-ATP) was 2.3 times greater than the slope of the ATP-direct method calibration line (563 RLU·L/ng-ATP). Moreover, an insignificant difference was observed between the slope of the ATP-filtration method calibration line in seawater (1293 RLU·L/ng-ATP) and its slope in freshwater (1347 RLU·L/ng-ATP), which confirms the deleterious effect of salt on the luciferase reaction. This result showed that a higher luminescence signal was obtained when the seawater ions were excluded from the ATP reaction. Theoretically, the high luminescence signal increases the sensitivity of the measurement.

The effect of sample volume was studied by measuring the luminescence signal and the calculated microbial ATP of 2 seawater samples with different volumes ranging between 1 and 30 mL (Table 2 and Fig. S4). The measured microbial ATP of Sample A ranged between 58.8 and 61.3 ng-ATP/L with 1.5% coefficient of variation, while the microbial ATP of Sample B ranged between 6.04 and 6.98 ng-ATP/L with 5% coefficient of variation. This result indicates that the sample volume has no effect on the measured microbial ATP and suggests the use of a higher sample volume for samples with low microbial ATP.

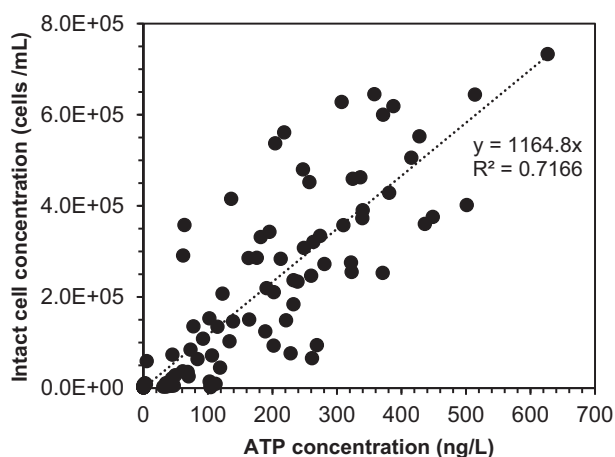
**Table 2**  
Measured luminescence signal and calculated microbial ATP of 2 seawater samples at different volumes between 1 and 30 mL.

Sample volume (mL)	Sample A		Sample B	
	Luminescence signal (RLU)	Microbial ATP (ng/L)	Luminescence signal (RLU)	Microbial ATP (ng/L)
1	13,543	58.8	2424	7.0
2	26,872	59.9	3845	6.3
3	39,971	59.9	6034	7.0
5	65,849	59.7	9082	6.5
10	132,821	60.5	18,683	6.8
15	201,605	61.3	27,362	6.7
20	269,623	61.6	32,712	6.0
30	396,881	60.5	52,280	6.5
Average (ng-ATP/L)		60.3		6.6
Standard deviation (ng-ATP/L)		0.9		0.3
Variation coefficient (%)		1.5		4.5

### 3.4. Correlation of ATP with the intact cell concentration

The microbial ATP of 100 seawater samples (North Sea) was measured using the ATP-filtration method, in which the microbial ATP concentration ranged from 0.5 to 670 ng-ATP/L. For the same set of samples, their intact cell concentration was also measured by flow cytometry (ICC-FCM) and ranged from  $1.1 \times 10^3$  to  $7.3 \times 10^6$  cells/mL. The correlation between the two parameters in seawater is presented in Fig. 5 ( $R^2 = 0.72$ ,  $Rho = 0.88$ ,  $P\text{-value} \ll 0.001$ ,  $n = 100$ ). A strong correlation was not expected as flow cytometry counts the total number of intact cells regardless of their activity, and microbial ATP measures the activity of cells regardless of their number. A correlation ( $R^2 = 0.69$ ,  $n = 200$ ) between the total cell count measured by flow cytometry and total ATP was reported in drinking water [14,23]. Furthermore, Hammes et al. [30] reported similar correlation ( $R^2 = 0.8$ ,  $P\text{-value} \ll 0.001$ ,  $n = 102$ ) between microbial ATP and ICC-FCM in freshwater. Van der Wielen and van der Kooij [45] also observed correlations ( $R^2 = 0.55$  (spring),  $R^2 = 0.82$  (winter),  $P < 0.01$ ,  $n = 48$ ) between total ATP and the total count of cells measured with epifluorescence microscopy in drinking water.

Based on the correlation found in this study, the average microbial



**Fig. 5.** Correlation between microbial ATP and intact cell concentration measured by flow cytometry in seawater ( $R^2 = 0.72$ ,  $Rho = 0.88$ ,  $P\text{-value} \ll 0.001$ ,  $n = 100$ ). An average microbial ATP per intact cell in seawater was derived from these data ( $8.6 \times 10^{-7}$  ng-ATP/cell).

ATP concentration per cell was estimated at  $8.6 \times 10^{-7}$  ng-ATP/cell. This is in agreement with the findings of Hamilton and Holm-Hansen [46], who used the ASTM method to measure the ATP content in seven selected cultures of marine bacteria. They reported that the average ATP content ranges from 5 to  $65 \times 10^{-7}$  ng-ATP/cell.

### 3.5. Comparing the ATP-filtration method with the ATP-direct method

Although the same reagents (lysis and detection reagent) were used to measure seawater in both the ATP-filtration and ATP-direct method, the protocol of each method is different. The main difference is that in the ATP-direct method, the reagents are added directly to the seawater; therefore, the matrix effects of the sample need to be taken into consideration by preparing a calibration line. In the ATP-filtration method, the microorganisms are collected on a membrane filter, and the microbial ATP is extracted on the filter itself. Table 3 summarizes the similarities and differences between the ATP-filtration method and the ATP-direct method.

#### 3.5.1. Pre-treated seawater in an SWRO plant

The microbial ATP concentration was measured through the pre-treatment train of the SWRO desalination plant using the ATP-filtration and ATP-direct methods (Fig. 6). In general, microbial ATP concentrations of both methods were comparable and ranged from 19 to 89 ng-ATP/L. Similar microbial ATP concentrations were measured (80 and 89 ng-ATP/L) in raw seawater using the ATP-filtration method and the ATP-direct method, respectively. A slight reduction (20%) in microbial ATP was noted after flocculation, while a significant reduction in microbial ATP was recorded (47%) after dual media filtration. After flocculation, microbial ATP measured using the ATP-direct method was lower than its concentration using the ATP-filtration method by approximately 20%. However, the average difference in the four measured samples across the pre-treatment train was  $< 10\%$ . A higher number of samples were studied in the next sub-section to accurately assess the differences between the two methods.

#### 3.5.2. Raw seawater

Microbial ATP measured by two different ATP methods were compared in 125 raw seawater samples collected from the North Sea and the Tasman Sea. It was found that both methods show comparable microbial ATP concentrations ranging between 1 and 1000 ng-ATP/L (Fig. 7). The measured microbial ATP concentration using the ATP-direct method is slightly lower (5%) than the concentration based on the ATP-filtration method. An ANOVA analysis showed a significant correlation ( $R^2 = 0.95$ ,  $P\text{-value} = 1.9 \times 10^{-80} \ll 0.001$ ,  $n = 125$ ) between the two methods. The significance of the correlation between the two methods was tested using a “t-test of 2 samples assuming equal variances”, which showed that the P-value (0.62) was much higher than alpha ( $\alpha = 0.05$ ) and t-Stat (0.5) was much lower than t critical two-tail (1.97), indicating that the correlation between the two methods is significant with 95% confidence. These results show that the measured microbial ATP concentrations by both the ATP-filtration and ATP-direct methods were comparable and correlated well.

### 3.6. Applications of the ATP-filtration method

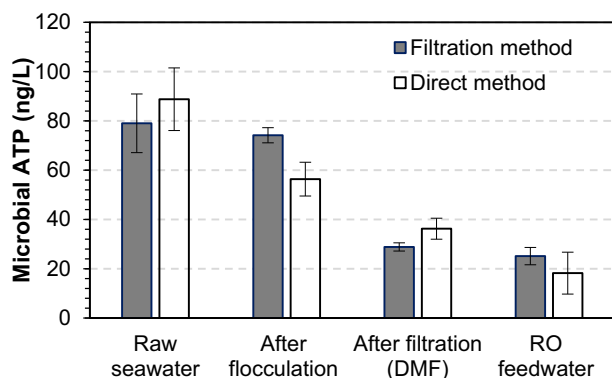
#### 3.6.1. Monitoring microbial ATP in a full-scale SWRO plant

The microbial ATP concentration was monitored along the treatment process of an SWRO desalination plant (with and without chlorination, 1 mg  $Cl_2/L$  in the intake). Samples were collected from raw seawater, after the first stage of dual media filtration (DMF1), after the second stage of dual media filtration (DMF2), after cartridge filtration, SWRO permeate and after remineralization.

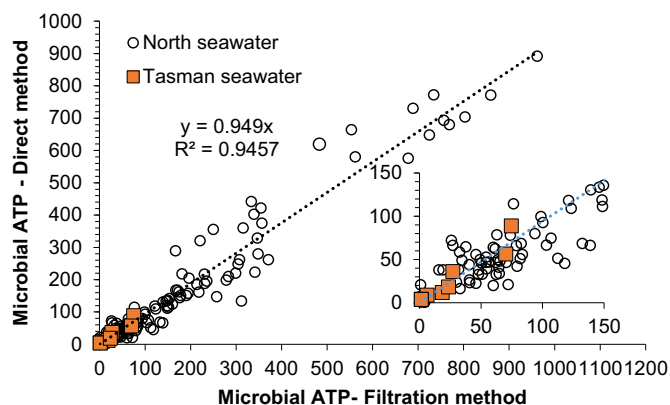
Before intermittent chlorination, microbial ATP concentration in the influent was 525 ng-ATP/L, and a significant reduction of microbial ATP ( $> 95\%$ ) was recorded through DMF1 incorporated with inline

**Table 3**  
Comparing the ATP-direct and the ATP-filtration methods.

	ATP-direct method	ATP-filtration method
Reagent for microbial ATP extraction	Water-Glo lysis reagent	Water-Glo lysis reagent
Reagent for light generation	Water-Glo detection reagent	Water-Glo detection reagent
Measurement	Microbial ATP = Total ATP – Free ATP	Microbial ATP only
Microbial ATP extraction	Direct - in the seawater sample	On a filter surface
Complexity (no. of steps)	Simple (2 steps)	Complex (4 steps)
Cost	Low	Moderate
Limit of detection	0.3 ng-ATP/L	< 0.06 ng-ATP/L
Matrix effect	Yes (pH, Mg <sup>+</sup> , Fe <sup>+</sup> )	No



**Fig. 6.** Comparison of microbial ATP concentration measured using the ATP-filtration method and the ATP-direct method for 4 samples collected during the pre-treatment of an SWRO desalination plant.



**Fig. 7.** Correlation between the ATP-filtration and ATP-direct methods for 125 seawater samples collected from North Sea water, and Tasman Sea water.

coagulation (3.8 mg-FeCl<sub>3</sub>/L) to 20 ng-ATP/L (Fig. 8). Insignificant ATP removal was measured (< 1%) through DMF2 and cartridge filtration. The remaining 4% of microbial ATP was removed through the SWRO membrane, in which microbial ATP after the 1st pass of the SWRO membrane and after remineralization were low (below 0.4 ng-ATP/L). The measured microbial ATP concentration in the SWRO permeate was 7 times higher than the LOD of the ATP-filtration method. The ATP-filtration method is very versatile because it can be used to measure microbial ATP concentration in seawater as well as SWRO permeate (freshwater) down to 0.06 ng-ATP/L.

When intermittent chlorination was applied, microbial ATP in the influent decreased to 27 ng-ATP/L (Fig. 8) due to the addition of 1 mg-Cl<sub>2</sub>/L in the intake pipe. Microbial ATP concentration after DMF1 was higher than the microbial ATP concentration in the influent, which could be due to biomass detachment during chlorination from the biofilm present in the media filter. The same observation was noted after DMF2 but at a lower magnitude, which may be because of a low

concentration of free chlorine reaching the second stage of DMF or the low biofilm formation in DMF2. Microbial ATP decreased from 22 to 10 ng-ATP/L after 5 μm pore size cartridge filtration step. Microbial ATP concentration after SWRO was also low (0.2 ng-ATP/L), which is 3 times higher than the LOD of the ATP-filtration method. However, a higher microbial ATP concentration was observed after remineralization compared to the measured concentration in the SWRO permeate. The high microbial ATP concentration after remineralization could indicate bacterial re-growth, which might occur due to nutrients originating from the added contaminated salts (calcium and fluoride).

The changes in microbial ATP clearly show the effect of added chlorination in the intake on each process along the SWRO pre-treatment train. It also demonstrates the applicability of the ATP-filtration method to monitor biological conditions through the pre-treatment of SWRO and after RO membrane systems. Moreover, this result suggests that measuring microbial ATP could be used to monitor the (biological) filtration performance of a dual media filter and might be useful for optimizing these processes.

### 3.6.2. Monitoring of single step media filtration

The ATP-filtration method was used to monitor the performance of a seawater media filtration in terms of backwashability and microbial removal through the filter. For this purpose, microbial ATP was measured in raw North Sea water (influent) and in the filtrate of the media filtration (effluent) within one filtration cycle (48 h).

In the filtrate of the seawater media filtration, microbial ATP ranged between 92 and 140 ng-ATP/L before backwashing and ranged between 150 and 200 ng-ATP/L within the first hour after backwashing (Fig. 9). This result indicates that 7 min of backwashing could be enough to remove most of biomass accumulated during 48 hour filtration cycle.

The removal of microbial ATP through the media filtration ranged between 65 and 85%. The microbial ATP concentration of the filtered seawater followed the same trend as the raw seawater. Microbial ATP in the raw seawater increased from 1050 to 3000 ng-ATP/L over day 1 and decreased to 1350 ng-ATP/L over day 2. Similarly, microbial ATP in the filtered seawater increased from 150 ng-ATP/L (after backwashing) to 450 ng-ATP/L over day 1 and decreased to 210 ng-ATP/L over day 2. The dissimilar trend of microbial ATP of day 1 and day 2 is attributed to the seawater temperature, which increased from 12 to 23 °C on day 1 and declined to 16 °C on day 2. Higher bacterial growth and production are commonly reported at higher seawater temperature [47].

This result may suggest the applicability of using microbial ATP measurements to monitor and optimize the performance of seawater media filtration.

### 3.6.3. Monitoring of bacterial growth potential in seawater

The ATP-filtration method was applied to monitor the bacterial growth potential using the indigenous microbial consortium. Samples across the treatment line of a pilot desalination plant (raw seawater, after media filtration and after ultrafiltration) were collected, pasteurized, inoculated with 10,000 intact cells/mL, incubated at 30 °C and bacterial growth was monitored daily over 5 days based on microbial ATP.



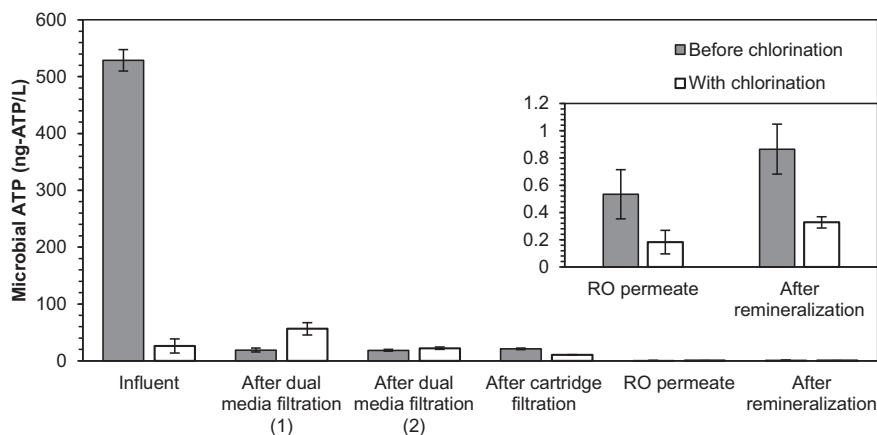


Fig. 8. Microbial ATP concentrations measured using the ATP-filtration method for samples collected through the treatment processes of a full-scale SWRO desalination plant. All data are plotted as average ± standard deviation (n = 3).

The maximum microbial ATP concentration was reached after 2 days starting from 2 ± 1 ng-ATP/L (Fig. 10). The maximum microbial ATP reached depended on the nutrients available in the seawater. The maximum growth of raw seawater was 327 ng-ATP/L and decreased to 183 ng-ATP/L after media filtration and to 160 ng-ATP/L after ultrafiltration. High reduction (44%) of bacterial growth potential and nutrient removal was achieved by media filtration, while additional 7% of bacterial growth potential reduction was achieved through ultrafiltration. The lower reduction of bacterial growth potential in the ultrafiltration might be because most of particulate organic matter was previously removed in the media filtration. This result is in line with the findings of Kim et al. [48] who observed 38% reduction of organic matter through seawater media filtration.

It can be seen from these results that the ATP-filtration method can be used to monitor bacterial growth in seawater.

4. Conclusions

- A new method was developed to measure microbial ATP in seawater by incorporating a filtration step to concentrate the sample and to overcome interference of salt. The measured microbial ATP concentration using the ATP-filtration method was comparable (± 5% difference, R<sup>2</sup> = 0.95, n = 125) to the concentration measured using the ATP-direct method in seawater.
- A very low limit of detection (0.06 ng-ATP/L, equivalent to 70 cells/mL) was obtained based on the ATP-filtration method, and is 3 times lower than the measured microbial ATP concentration in an SWRO permeate.
- Microbial ATP concentration in North Sea water samples correlated

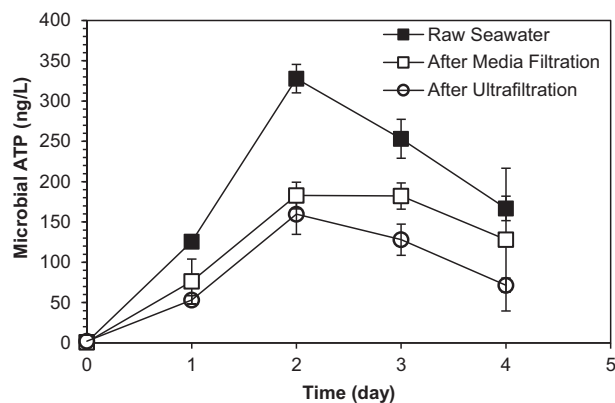


Fig. 10. Monitored bacterial growth potential based on microbial ATP using the ATP-filtration method. Samples were collected from raw seawater (■), after media filtration (□) and after ultrafiltration (○) of a pilot-scale plant (the Netherlands). Data of Day 0 is the initial microbial ATP concentration after inoculation.

with intact cell concentration measured by flow cytometry (R<sup>2</sup> = 0.72, Rho = 0.88, P << 0.001, n = 100). The average microbial ATP per marine bacterial cell was 8.59 × 10<sup>-7</sup> ng-ATP/cell.

- The ATP-filtration method was applied to measure microbial ATP along the pre-treatment and permeate of an SWRO desalination plant. In the SWRO plant, significant reduction of microbial ATP (> 95%) was recorded through the first stage of dual media filtration (with inline coagulation 3.8 mg-FeCl<sub>3</sub>/L). A low microbial ATP

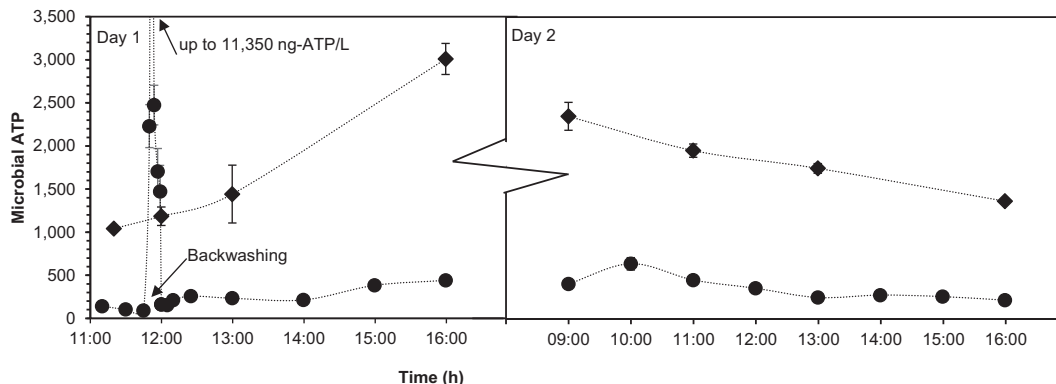


Fig. 9. Monitored microbial ATP concentrations before and after seawater media filtration over one filtration cycle. Symbols: (◆) Raw seawater, (●) Filtered seawater.

concentration was measured in the SWRO permeate (0.2 ng-ATP/L).

- The removal of microbial ATP through media filtration ranged between 65 and 85% and 7 min of backwash time was sufficient to remove the accumulated microorganisms during 48 h of filtration.
- The ATP-filtration method was employed to monitor bacterial growth potential across the pre-treatment train of a seawater pilot plant, in which the reduction in bacterial growth potential was 44 and 7% through the media filtration and ultrafiltration, respectively.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.desal.2018.11.020>.

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