Monitoring Adenosine Triphosphate and bacterial regrowth potential in Seawater Reverse Osmosis Plants

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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 through pre-treatment trains of seawater reverse osmosis (SWRO) plants as the level of detection is significantly lower than required to prevent biological fouling in reverse osmosis membrane 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.
1. Introduction

Controlling biological fouling in 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 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 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 (BDOC) [7] and biomass production potential (BPP) [8, 9]. Weinrich et al., 2016 [10] detected more biofouling (using a flat sheet RO membrane) when the AOC concentration of the feed water increased from 30 to 1,000 µg.L⁻¹. In addition, differential pressure increased from 3.5 to 6.2 bar during 9 days 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 counting [6, 11-13], flow cytometry [14], 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., (2013) [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. (2011) [18] adapted the AOC-bioluminescence freshwater method for seawater by using a specific strain of bioluminescent marine bacteria, Vibrio harveyi. Jeong et al., (2013) [4] found a strong correlation between the number of another single bioluminescent strain, Vibrio fischeri and the bioluminescence signal (in the range of 10⁵ to 10⁶ 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 hour and 1-3 days, 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 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 heterotrophic plate count (HPC), total direct count by microscope (TDC), flow cytometry (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 (>10⁵ 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 feed water [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 et al., (2011) [32] suggested diluting seawater with demineralised water to an electrical conductivity of 4 mS/cm (2.5 mg/L) 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 bio-filter for seawater with reported high ATP concentrations. LOD using the freshwater reagent kit (BacTiter-Glo) in seawater was investigated in our group to be 50 ng.L\(^{-1}\) (2×10^5 cells.L\(^{-1}\)). Van Slooten et al., (2015) [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 limit of detection necessary in seawater reverse osmosis systems, the lowest threshold AOC concentration to avoid biofouling in RO membranes was used. Hijnen et al., (2009) [27] reported 1 µg-acetate.L\(^{-1}\) in RO feed water 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., (2010) (1 µg-acetate.L\(^{-1}\) = 1×10^4 cells.mL\(^{-1}\)) [35]. 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 cells.mL\(^{-1}\) = 2.5 ng.L\(^{-1}\) found in our research group.

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

1. Verifying the luminescence signal and stability of the new detection reagents in seawater.
2. Calibration curve and the limit of detection of the measurement.
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.

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.9 ± 0.1</td>
</tr>
<tr>
<td>TDS (g.L(^{-1}))</td>
<td>32.5 ± 0.8</td>
</tr>
<tr>
<td>Conductivity (mS.cm(^{-1}))</td>
<td>52.6 ± 1.2</td>
</tr>
<tr>
<td>Total bacterial count (cells.mL(^{-1}))^*</td>
<td>1.2 ± 0.48 ×10(^{6})</td>
</tr>
<tr>
<td>TOC (mg.C.L(^{-1}))</td>
<td>1.28 ± 0.85</td>
</tr>
<tr>
<td>UV(_{254}) (cm(^{-1}))</td>
<td>0.045 ± 0.009</td>
</tr>
</tbody>
</table>

*measured with FCM

2.2 Preparation of artificial seawater (ASW)

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\(_2\)O, 4.0 g.L\(^{-1}\) Na\(_2\)SO\(_4\), 1.54 g.L\(^{-1}\) CaCl\(_2\).2H\(_2\)O, 0.74 g.L\(^{-1}\) KCl, 0.21 g.L\(^{-1}\) NaHCO\(_3\), and 0.002 g.L\(^{-1}\) Na\(_2\)CO\(_3\)). 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.

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., USA) was added directly to 100 µL of the seawater sample in a 1.5 mL micro centrifuge 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., USA) 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., USA) 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 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 hour) and 15 mL was transferred into 30 mL AOC-free vials (heated in an oven furnace for 6 hours 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 1×10⁶ intact-cells/mL (measured by flow cytometry) in each vial. The samples were incubated at 30 °C. The bacterial growth of the seawater sample was monitored for 5 days using the ATP protocol described in Section 2.3.

2.5 Determination of limit of detection (LOD)

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 [37]. 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 \( \text{LOD} = \sqrt{\text{LOD of Free ATP}^2 + \text{LOD of Total ATP}^2} \).

![Figure 1. Treatment scheme of the tested SWRO desalination plant in Australia and the locations of the collected samples.](image)

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 to BacTiter-Glo (combined freshwater reagent). The ATP Water-Glo reagent showed higher luminescence signal (> 20x) compared to that obtained with BacTiter-Glo, when applied in seawater (Fig 2a). 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 to that of BacTiter-Glo (2,263 RLU) in artificial seawater (35 g.L⁻¹). The high luminescence signal and low background luminescence of the ATP Water-Glo reagents suggest that the new reagents provide more sensitivity than BacTiter-Glo (freshwater reagent) when used in seawater.

![Figure 2: (a) 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) Stability of ATP Water-Glo and BacTiter-Glo reagents in artificial seawater maintained at 4 °C and 23 °C.](image)
reagents over time at different storage temperatures. (c) Stability of luminescence signal over time for a seawater sample measured with ATP Water-Glo reagent.

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 days at 23 °C, whereas the activity of BacTiter-Glo dramatically decreased within the first day (Figure 2b). The stability of the luminescence signal was also tested, and was stable for 40 seconds after the addition of ATP Water-Glo reagent to the seawater sample (Fig 2c). 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 Calibration and limit of detection determination

3.2.1 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 artificial seawater, 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⁻¹ATP.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 artificial seawater 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. 3). However, preparing artificial seawater with similar properties to real seawater is very tedious. Therefore, the use of sterilized seawater at 121 °C is recommended.

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.).

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

<table>
<thead>
<tr>
<th>Calibration line properties</th>
<th>Real seawater - standard addition</th>
<th>Pasteurized seawater - (70 °C)</th>
<th>Sterilized seawater - (121 °C)</th>
<th>Filtered seawater - (0.1 µm)</th>
<th>Artificial seawater</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibration line properties</td>
<td>Slope of the calibration (RLU.ng⁻¹ATP.L)</td>
<td>559.9</td>
<td>559.5</td>
<td>556.7</td>
<td>557.9</td>
</tr>
<tr>
<td>Regression coefficient (R²)</td>
<td>0.998</td>
<td>0.992</td>
<td>0.999</td>
<td>0.996</td>
<td>0.999</td>
</tr>
<tr>
<td>Intercept point with y-axis</td>
<td>Average (RLU)</td>
<td>65,365</td>
<td>10,611</td>
<td>661</td>
<td>5,996</td>
</tr>
<tr>
<td></td>
<td>Standard deviation (RLU)</td>
<td>742</td>
<td>82</td>
<td>20</td>
<td>249</td>
</tr>
<tr>
<td></td>
<td>Variation coefficient (%)</td>
<td>1.2</td>
<td>1.5</td>
<td>4</td>
<td>4.2</td>
</tr>
</tbody>
</table>
3.2.2 Limit of detection

The limit of detection (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 were 0.2 and 0.2 ng.L\(^{-1}\), respectively (Table 3) [37]. The combined LOD of Microbial ATP was 0.3 ng.L\(^{-1}\), which is approximately 1,200 cell.ml\(^{-1}\) (using the correlation found within our group). The reported LOD of freshwater ATP methods are in the range between 0.05 and 5.1 ng.L\(^{-1}\) [20, 29, 38, 39].

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

<table>
<thead>
<tr>
<th></th>
<th>Total ATP</th>
<th>Free ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average blanks (RLU)</td>
<td>584</td>
<td>191</td>
</tr>
<tr>
<td>Standard deviation (RLU)</td>
<td>16</td>
<td>11</td>
</tr>
<tr>
<td>LOD (ng.L(^{-1}))</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>LOD of the method</td>
<td>0.3</td>
<td></td>
</tr>
</tbody>
</table>

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 feed water. 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., (2009) [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\(^4\) cells.ml\(^{-1}\) [35]. The LOD of the direct ATP method in seawater (0.3 ng.L\(^{-1}\), 1,200 cells.ml\(^{-1}\)) is approximately 8 times lower than the reported threshold concentration (1×10\(^4\) cells.ml\(^{-1}\)) suggesting that ATP can be used to monitor bacterial growth potential in SWRO feed water.

3.3 Application of the Microbial ATP method

3.3.1 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 ng.L\(^{-1}\) to 1,037 ng.L\(^{-1}\) with the lowest concentration observed during the winter months as shown in Fig. 4. It is not unlikely that micro-algae were also lysed by the Water-Glo lysis reagent. In the winter, Microbial ATP ranged between 20 ng.L\(^{-1}\) 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 cells.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.
3.3.2 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 SWRO desalination plant (Fig. 5), which is relatively low compared to 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, dual media filter 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).

![Graph showing Microbial ATP measurement through the RO pre-treatment processes of an SWRO desalination plant in Australia.](image)

**Figure 5:** Microbial ATP through the RO pre-treatment processes of an SWRO desalination plant in Australia.

Microbial ATP measurement was also applied to monitor the bacterial growth potential across the pre-treatment processes of an SWRO plant (Fig. 6a). 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 days. 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. 6b), 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., 2011 [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 a 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.

Figure 6: (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.
- 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 limit of detection 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 ng.L⁻¹ 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.

5 Acknowledgments

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6 References


