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Evaluating adenosine triphosphate bioluminescence for biomonitoring in potable water systems

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ABSTRACT

The application of adenosine triphosphate (ATP) bioluminescence technology as a rapid microbiological activity monitor within a potable water system has been demonstrated. ATP measurements were taken pre- and post-infrastructure improvements within a watershed and throughout two potable water systems from source to tap. A reduction in ATP, as measured by relative light units, was identified post-infrastructure improvements. Peak ATP values for the watershed were found within the reservoirs. The treated source water remained biologically stable throughout the distribution system, with peaks attributed to nitrification. A mathematical model for predicting microbial ATP using pH, temperature, and alkalinity, was developed for the watershed studied, with an adjusted R^2 of 0.84 at a 95% confidence level. Overall, ATP bioluminescence technology was found to be a suitable candidate for rapid microbiological activity testing in drinking water systems; however, technological limitations remain with respect to reproducibility that should be addressed prior to full-scale implementation.

Key words | ATP, biomonitoring, distribution system, drinking water, modelling, watershed

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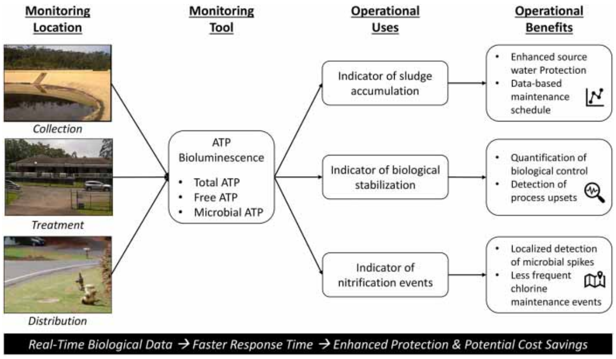
HIGHLIGHTS

- Microbial ATP could be used to detect when reservoir maintenance is required.
- Microbial ATP is a useful real-time tool for detecting isolated nitrification events within a chloramine system.
- Microbial ATP levels are site specific and predictable using common water quality parameters.
- Microbial ATP spikes can identify a breach in distribution system security.

GRAPHICAL ABSTRACT



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INTRODUCTION

The methods used today to monitor microbial activity in a water system focus on the detection of microbial activity through time-intensive, culture-dependent tests such as heterotrophic plate counts (HPC). There is a general consensus for the need of fast and accurate technologies for biomonitoring in water (Delahaye *et al.* 2003; Berney *et al.* 2008; Vital *et al.* 2012), with adenosine triphosphate (ATP) bioluminescence being investigated and promoted as a potential indicator and estimator of microbial activity since the 1960s (Hammes *et al.* 2010).

The ATP cycle is the primary energy production and carrier cycle for living cells (Hammes *et al.* 2010). ATP is converted to adenosine diphosphate (ADP) through the breaking of a chemical bond, releasing energy and a phosphate group. The released energy is used for anabolic processes, such as cell growth and reproduction processes. To complete the cycle, a bond is formed between a lone phosphate group and an ADP molecule, requiring energy, to make an ATP molecule. The required energy originates from catabolic processes, such as the breakdown of polysaccharides into monosaccharides. This cycle is used by cells to store and release the chemical energy needed to survive and function. ATP in the environment is the summation of both active (live; intracellular) and inactive (dead; extracellular) cells (Thore *et al.* 1975). Thus, an important distinction is made between extracellular ATP and intracellular ATP, where the extracellular ATP levels in a sample are the background (or baseline) for intracellular ATP measurements. Typically, an abundance of intracellular ATP signals high microbiological activity while an absence of intracellular ATP signals low microbiological activity.

ATP bioluminescence technology relies on the ATP cycle and the firefly reagent *luciferase* to produce light via a chemical reaction (Davidson *et al.* 1999). The intensity of the light emitted by the reaction is proportional to the amount of ATP in the sample. ATP bioluminescence analysis only requires about 1 min per sample for results to be available, allowing for real-time corrective measures in cases of contamination (Lee & Deininger 1999). Extensive research has been undertaken into correlation models between ATP and HPC values and ATP and total cell count (TCC) values with significant success (Lee & Deininger 1999; Ikonen *et al.* 2013; Van Nevel *et al.* 2017). Furthermore, research has shown that the addition of ATP bioluminescence for biomonitoring can reduce false-positive HPC results, as well as improve detection of viable but nonculturable state bacteria which cannot reproduce but maintains infectious ability (Ghazali *et al.* 2010; Vital *et al.* 2012; Kong *et al.* 2015). Although the technology has various limitations, recent studies on the reliability of ATP bioluminescence as a microbiological indicator for different aspects of the drinking water industry have shown favorable results (Vrouwenvelder *et al.* 2008; Vang *et al.* 2014; Van Nevel *et al.* 2017).

The majority of ATP bioluminescence research has been conducted outside of the United States in water systems that do not employ secondary disinfection. These studies found that distribution system microbial activity is stable (Vital *et al.* 2012), seasonal effects on microbial activity can be seen in surface waters (Delahaye *et al.* 2003; van der Wielen & van der Kooij 2010), flow rates can affect ATP concentrations in water (Lehtola *et al.* 2006; Douterelo *et al.* 2019), free ATP can trigger regrowth due to an increase in dissolved phosphate (Nescerecka *et al.* 2016), and ATP levels decrease as water travels through a distribution network (van der Wielen & van der Kooij 2010). Thus, a gap in the literature exists for elucidating the behavior of ATP within the United States where secondary disinfection is required for potable water systems. To address this gap in knowledge with regard to the use and effectiveness of microbial ATP, this paper (a) investigated how ATP behaves in a surface water collection and conveyance system to determine how microbial ATP could be used in the future to enhance source water protection and cost-effective operations, (b) characterized the microbial ATP levels of a water system from source to tap to evaluate the operational benefits of ATP bioluminescence, and (c) examined the sensitivity of microbial ATP at detecting differences in microbiological activity between two distribution systems with different secondary disinfectants and within each distribution system (different locations). The following sections describe the research methodology used, present the findings of the completed data analysis, and summarize the study's conclusions.

MATERIALS AND METHODS

The research reported herein was conducted with the assistance of the county of Maui department of water supply (Kahului, HI). The Hawaiian upper elevation water system consists of the Waikamoi Rainforest watershed, the Olinda water treatment plant (WTP), and the Upper Kula distribution system. The Olinda WTP utilizes coagulation with aluminum chlorohydrate and ultrafiltration membrane treatment to treat water prior to disinfection with chloramines (primary and secondary). The aged Waikamoi water collection system was rehabilitated through improvements completed to a flume and two 15 million-gallon (MG) reservoirs. Microbial ATP was monitored within the Waikamoi water collection system in the wet (November–April) and dry (May–October) seasons prior to the rehabilitation work (2012–2014) and post the rehabilitation work (2017–2018). Microbial ATP levels were also monitored throughout the treatment train at the Olinda WTP and the Upper Kula distribution system between 2017 and 2018. During this time, the Hawaiian middle elevation WTP (Pi'iholo) and distribution system (Lower Kula) were also monitored using microbial ATP. The Pi'iholo WTP utilizes coagulation with aluminum chlorohydrate and dual-media filters to treat water, with a portion of this treated stream being sent to a granular activated carbon system. The blended stream from the dual-media filters and carbon system is then disinfected with chlorine (primary and secondary).

Sample locations and frequency

Microbial activity was measured throughout the middle elevation and high elevation water systems of Upcountry Maui (Figure 1), with monitoring occurring at a frequency of four data sets (on average) per year. Twelve locations within the watershed were monitored, eight pipelines (mixture of aluminum, PVC, and cast iron pipes) and four reservoirs (two 15 MG (56,781 cubic meters) and two 50 MG (189,271 cubic meters)). The four reservoir sampling points were considered laminar (slow) flow, with the remaining eight sampling points considered turbulent (fast) flow. For the Olinda WTP (chloramine system), the sampling locations were raw water, settled water, decant water, UF filtrate, and finished water. The Upper Kula distribution system sampling locations have been summarized in Figure 2. The eight sample points represent a mixture of tanks, standpipes, and hydrants within the distribution system with Omaopio Tank being the first and Kanaio Tank being the last distribution system tanks. The sampling locations for the Pi'iholo WTP (chlorine system) were raw water, flocculated water, dual-media filtrate, GAC filtrate, and finished water. Figure 3 graphically represents the eight sampling points within the Lower Kula distribution system which include a mixture of standpipes and hydrants, as well as a tank.

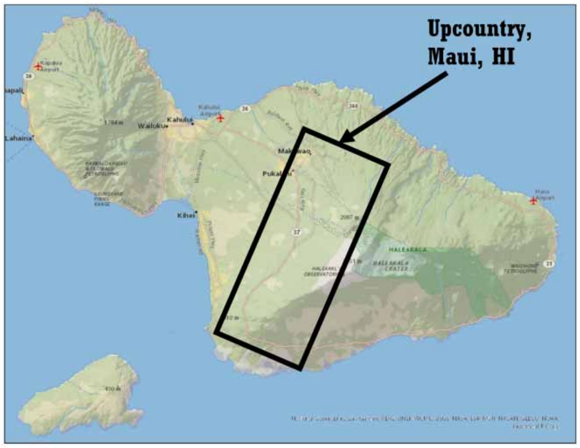

Figure 1 | Waikamoi watershed system sample locations.

Equipment, reagents, and sample preparation

Intracellular ATP (microbial ATP) monitoring was conducted using a 3 M (St Paul, MN, USA) luminometer (NG3) with free ATP (AQF100) and total ATP (AQT200) kits. Duplicate grab samples were taken at each monitoring site in 250 mL beakers for *in situ* free and total ATP analysis. Microbial ATP was calculated by subtracting the free ATP from the total ATP measured at each site. Additional water quality parameters monitored during this study included pH, temperature, and alkalinity. Water quality analyses were conducted in accordance with *Standard Methods for the Examination of Water and Wastewater* (Baird *et al.* 2017).

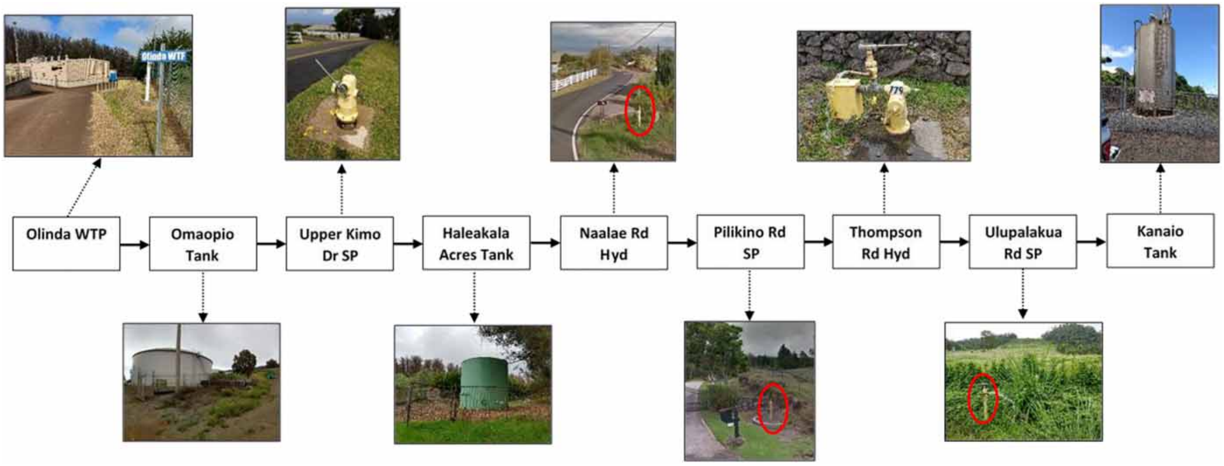

Figure 2 | Upper Kula distribution system sample locations.

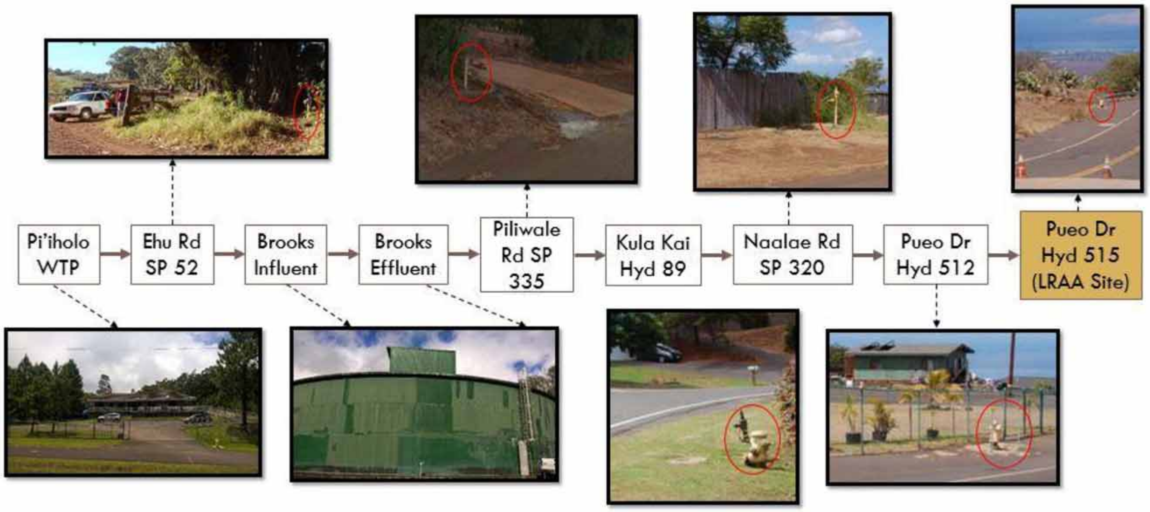

Figure 3 | Lower Kula distribution system sample locations.

Data analysis

Microbial ATP data were analyzed using Microsoft® Excel to determine locational means and standard deviations, as well as means and standard deviations for each system (watershed, WTP, and distribution systems). To address the first objective, the microbial ATP data sets were split between pre-rehabilitation and post-rehabilitation time periods, with mean dry season and mean wet season values calculated for the watershed. The watershed data were further delineated by flowrate type, with graphs generated to show the differences in microbial ATP levels based on flowrate, season, and rehabilitation efforts. In addition, a microbial ATP model was developed using the watershed post-rehabilitation mean microbial ATP, pH, temperature, and alkalinity data for each site. The second and third objectives were investigated using box-and-whiskers plots generated for the two potable water systems, with additional detailed plots generated for each site within the chlorine and chloramine distribution systems.

RESULTS AND DISCUSSION

Watershed monitoring findings

An overall reduction in the microbial activity of at least 95% was identified for both the dry and wet season after the infrastructure improvements, as measured through microbial ATP system averages. In addition, microbial activity for this watershed was identified to be higher during the dry season both before and after the infrastructure improvements. The pre- and post-rehabilitation period data sets were then categorized based on location (pipelines, 15 MG (56,781 cubic meters) reservoirs, and 50 MG (189,271 cubic meters) reservoirs) to investigate the effect of flow velocity on microbial activity. The segregated data were graphed using a box-and-whiskers plot and included in Figure 4. The results show that for both the pre- and post-rehabilitation periods, the pipelines exhibit the lowest microbial activity out of the three identified flow velocities. For the pre-rehabilitation period, the highest microbial activity was detected in the smaller reservoirs and for the post-rehabilitation period, the highest microbial activity was detected in the larger reservoirs.

Analysis of the collected microbial data indicates that infrastructure improvements, season, and flow velocity play important roles in the level of detectable microbial activity in water using microbial ATP. With respect to the watershed monitoring findings, the overall reduction in the microbial activity post-rehabilitation was theorized to be due to the removal of the accumulated sludge within the smaller reservoirs. The reservoirs within the water system were built to provide necessary storage for drought conditions but also serve as sedimentation basins, with the smaller reservoirs receiving partially clarified water from the larger reservoirs for further clarification prior to conveyance to the WTP. Sludge accumulation in raw water reservoirs reduces drought storage capacity and appears to enhance biological activity, likely from the formation of biofilms, which in excessive quantities could increase the probability of infectious diseases being present if not removed in a routine and timely manner. Since only the 15 MG (56,781 cubic meters) reservoirs were cleaned out during the rehabilitation work, the two 50 MG (189,271 cubic meters) reservoirs now exhibit the most microbial activity. Microbial ATP could eventually be used to indirectly track sludge accumulation within raw water reservoirs to provide utilities with a tool for determining when maintenance is required.

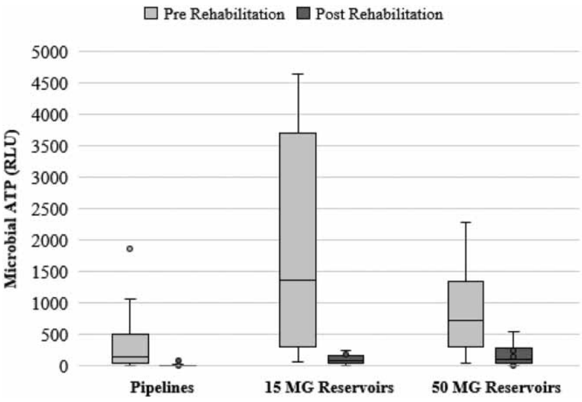

Figure 4 | Watershed ATP monitoring results.

For this watershed, the dry season exhibited higher microbial activity both pre- and post-rehabilitation which is hypothesized to stem from lower flow velocities through the water collection and conveyance system. Based on the results from Figure 4, flow velocity seems to have an inverse relationship with microbial activity. The faster or more turbulent the flow (pipelines), the less microbial activity detected in the water. The slower or more laminar the flow (reservoirs), the more microbial activity detected in the water. This could be due to differences in biofilm structures and density, as well as nutrient mass transfer rates, under low and high flowrates (Lehtola *et al.* 2006; Liu *et al.* 2016; Douterelo *et al.* 2019). Therefore, for this raw water collection and conveyance system, some microbial control is achieved hydraulically without the need of chemical disinfectants which provides cost savings to the utility.

A novel mathematical model for predicting microbial ATP utilizing water quality parameters was developed using the regression tool in Microsoft® Excel. Equation (1) represents the empirically derived mathematical relationship between microbial ATP (RLU) and pH, temperature (°C), and alkalinity (mg/L as $CaCO_3$) for the post-rehabilitation watershed data set. Based on *p*-values for the variables in this model, it appears that pH had the greatest influence on microbial ATP levels, followed by alkalinity and then temperature. The analysis of variance (ANOVA) provided an R^2 of 89%, and an adjusted R^2 of 84%, at a 95% confidence level. Modeled ATP values versus actual ATP values

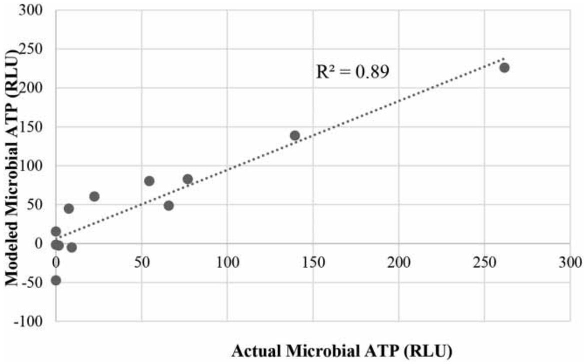

Figure 5 | Watershed actual ATP vs. modeled ATP results. *Note*: Data ranges – pH: 4.34–6.75 | Temperature, °C: 17.1–19.7 | Alkalinity, mg/L as $CaCO_3$: 0.97–5.80.

have been plotted to graphically represent the accuracy of the model (Figure 5).

$$\begin{aligned}\text{Microbial ATP (RLU)} = {} & (277 * \text{pH}) \\ & + (54.8 * \text{Temperature}) \\ & - (137 * \text{Alkalinity}) - 2,065 \qquad (1)\end{aligned}$$

Several models correlating microbial ATP with flow cytometry or HPC data have been developed (Hammes *et al.* 2010; Ikonen *et al.* 2013; Van Nevel *et al.* 2017). Existing models which focus on correlating water quality parameters, such as pH and temperature, to microbial activity typically do not use microbial ATP as the microbial activity parameter (Ikonen *et al.* 2013). Research correlating water quality parameters to microbial ATP was found to be minimal, with one study identifying the significance of temperature on microbial ATP levels (van der Wielen & van der Kooij 2010). The developed empirical model exemplifies the complex relationship between microbial ATP and water quality, with both the pH and buffering capacity of the water playing important roles. The model was used to predict the microbial activity for the pre-rehabilitation watershed data and the distribution system data with minimal success. The site specificity of the model is understood to be due to differences in microbial communities among the systems affecting the accuracy of the developed model (Hammes *et al.* 2010; Buysschaert *et al.* 2018). Models relating microbial ATP to common water quality parameters in watershed and distribution systems can provide utilities with a cost-effective way to predict the microbial activity of water within their system based on parameters already monitored.

Source to tap monitoring findings

A box–and-whiskers plot with post-rehabilitation watershed, WTP, and distribution system data was developed to evaluate the microbial activity of the chloramine water system, from source to tap. Figure 6 shows that microbial activity gradually increases as the source water travels from the pipelines to the reservoirs in the watershed, then is significantly decreased and stabilized by the WTP processes. A significant drop in the microbial activity from the larger reservoirs to the WTP raw water was identified for the system. The microbial activity of the treated water closely resembles the microbial activity of the distribution system, indicating a microbiologically stable system. There were more identified microbial ATP outliers in the distribution system than the treated water.

With respect to the source to tap monitoring findings, a significant decrease in microbial ATP was identified from the larger reservoirs to the WTP raw water sampling points (Figure 6). This is believed to be due to the flow conditions of the WTP raw water reservoir, the water no longer in contact with accumulated sludge, as well as the introduction of the decant water stream to the headworks of the WTP. The decant water includes backwash as well as clean in place water, which contains neutralized sulfuric acid and bleach which may act as unintended microbial activity controls. The higher instances of outliers for the distribution system data are suspected to be due to nitrification, which was corroborated by distribution system pH and nitrate data. Looking at microbial ATP from source to tap

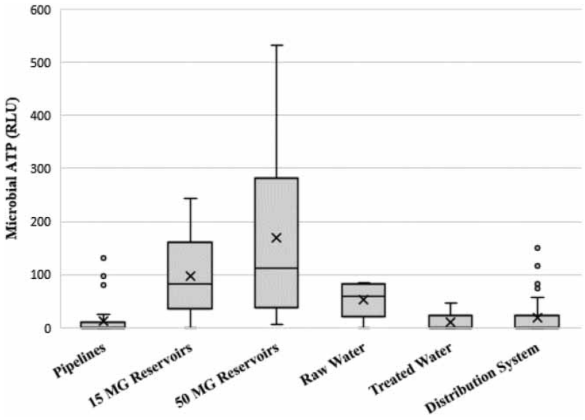

Figure 6 | Source to tap ATP monitoring results.

provides utilities with a quick snapshot of the microbial activity levels in their raw water collection system, the microbial control performance of their WTP processes, and the identification of potential microbial activity spikes within their distribution system.

Chloramine vs. chlorine system findings

A box-and-whiskers plot comparing microbial ATP levels for the raw water, treated water, filtered water, disinfected water, and distribution system water of a chloramine and chlorine system is presented in Figure 7. The chloramine system water was found to consistently exhibit higher microbial activity and variability than the chlorine system water. The chloramine system distribution water also had more cases of outliers within the data than the chlorine system distribution water. The distribution system data were expanded to show the microbial ATP results at each of the eight sampling sites for both systems to further investigate the outliers (Figures 8 and 9). While the mean microbial ATP values throughout both systems were below 40 relative light units (RLUs), the actual average value and variability was system specific and site specific, with higher average values and variability found within the chloramine system sampling sites.

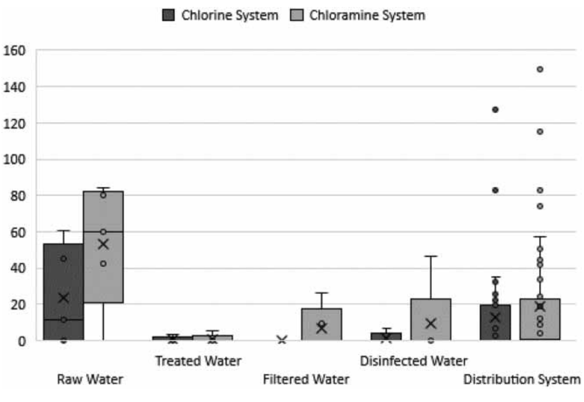

Figure 7 | Chloramine vs. chlorine system ATP monitoring results.

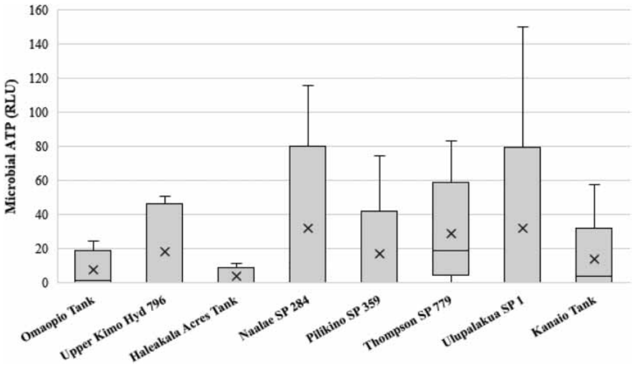

Figure 8 | Chloramine distribution system ATP monitoring results.

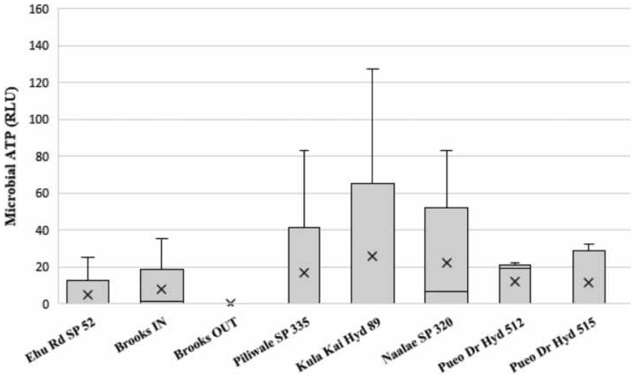

Figure 9 | Chlorine distribution system ATP monitoring results.

With respect to the chloramine vs. chlorine system findings, the higher microbial activity detected within the chloramine system is theorized to be caused by higher instances of microbial regrowth from nitrification due to the presence of ammonia. The distribution system data (Figures 8 and 9) further elucidates the potential relationship between flow and microbial activity. Based on the data gathered, the hypothesis for distribution systems is that stagnant flow and high flow conditions both result in lower microbial activity than low flow conditions. This phenomenon may be due to the depletion of the substrate in the water to support microbial activity (stagnant flow) or the formation of thin and dense biofilms which resist washout and dissolution of microbes into the bulk solution (high flow) (Lehtola *et al.* 2006; Liu *et al.* 2016; Douterelo *et al.* 2019). Microbial ATP monitoring of the distribution system could aid utilities in identifying spikes in microbial activity (nitrification or contamination via leak or other) in a timely manner to enhance service and water quality. For example, utilities that use chloramines could approach nitrification issues in a more localized manner, thereby potentially reducing the frequency of full system chlorine maintenance events, which require notices to be sent to customers and could potentially negatively impact the system corrosion rates.

CONCLUSIONS

This study investigated the use of microbial ATP as a real-time biomonitoring tool within a water system in a Hawaiian island where secondary disinfection is practised. Data analysis yielded the following key takeaways:

- Microbial ATP reductions of at least 95% were identified post-infrastructure rehabilitation within the Waikamoi Rainforest collection system. Post-rehabilitation microbial activity within the raw water reservoirs, as a measure through ATP, was found to be increasing with time. Thus, microbial ATP could be used as a tool in the water industry to manage these reservoirs and detect when maintenance (i.e. removal of sludge) is required with the development of a threshold ATP value for the system.
- Microbial ATP was found to be higher during the dry season and under laminar flow conditions within the watershed. Since seasonal changes and flow velocity can significantly impact microbial ATP values, proper implementation of this technology as a biomonitoring tool would likely require the development of a baseline for the system to be able to detect true anomalies or spikes in microbial activity. The ability to detect real-time microbial ATP spikes within a watershed can provide enhanced security and protection of our raw water sources against manmade pollution.
- Unlike most models in literature which utilize HPC or TCC, this study developed a novel model that correlates microbial ATP to pH, temperature, and alkalinity. The ANOVA analysis of the model yielded an R^2 of 89%, and an adjusted R^2 of 84%, at a 95% confidence level. The model was found to be site specific, likely due to the differences in the microbial ecosystem. Nevertheless, the model provides insight into the microbial activity of the watershed studied by monitoring three water quality parameters.
- Microbial ATP was found to be sensitive enough to detect the differences in microbial activity between a chlorine and chloramine distribution system. Furthermore, differences in microbial ATP throughout the distribution systems were identified, likely associated system hydraulics (flow velocities and dead ends). Thus, microbial ATP could become a useful real-time tool for detecting isolated nitrification events within a chloramine system, as well as determine when a full system chlorine maintenance event is needed. Regardless of disinfection, chemical, microbial ATP monitoring could enhance the protection of our potable water system against contamination from pipe leaks, backflows, and other pollution routes.

The results from this study indicate that ATP bioluminescence could be used as a microbial activity screening parameter if a water system baseline is established. As it stands today, technological limitations render the technology unreliable in terms of detecting a specific pathogen or predicting its presence, but it can readily detect changes in microbial activity. Thus, this technology may be advantageous as a tool to identify potential microbial activity changes within a system for further investigation. Once suspect locations are identified, current microbial monitoring methods (HPC or TC) or new methods such as flow cytometry could be used to determine if the change in microbial activity level is benign or pathogenic.

As the cost of ATP bioluminescence continues to drop, this new tool could help reduce laboratory costs for utilities as well as improve the level of protection and response time for potential pathogenic contamination in potable water systems. Once a robust ATP baseline is established, models using basic water quality parameters could also be used to further decrease the laboratory costs for utilities. ATP bioluminescence technology shows promise and utility for the water industry but would require regulatory guidelines for proper implementation in the future.

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(DWRG) and the Pi'iholo water treatment plant operations staff. Specifically, we would like to thank Tony Linder and James Landgraf from DWS and DWRG alumni Dr Andrea Netcher and Samantha Myers-O'Farrell for their assistance with data collection throughout the years. This material is based upon work supported by the National Science Foundation Graduate Research Fellowship under Grant No. 1144246. Any opinion, findings, and conclusions or recommendations expressed in this material are those of the authors(s) and do not necessarily reflect the views of the National Science Foundation, nor of the funding agency supporting this work.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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