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Effective Microbial Monitoring Systems

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Control of microbial contamination is one of the most critical functions of a water technologist's job. Without proper preventative measures, cooling towers can rapidly be fouled by uncontrolled microbial growth. Proper dosing with biocides is critical in maintaining an effective biocide concentration while limiting the expenses associated with overdosing. Overdosing biocides can lead to decreased cycle numbers and increased frequency of blow-down, which can dramatically increase the usage of biocides and in turn, decreases the profit margin in a tower-maintenance contract.

Maintaining control over microbial contamination requires an accurate assessment of the amount of contamination present. While traditional methods (i.e. dip-slides) are acceptable for monitoring the level of microbial contamination, they suffer from a few shortcomings:

(1) the methods require 24-96 hours for results;
(2) only a small, variable percentage of the organisms present in a water system will grow on standard growth media;
(3) the organisms that are really dangerous to industrial water systems (e.g. SRB's, Nitrobacter, Legionella) are not detected by agar films or dipslides.

Methods based on the detection of Adenosine Triphosphate (ATP) have been used in the water treatment industry for over a decade. ATP methods have several advantages over traditional plating methods in that they require less than a minute to perform and signal is obtained from every cell in the system.

Total ATP tests measure both the ATP contained within living cells as well as ATP floating free in the water. Free ATP tests measure only the ATP floating free outside of living cells. These two types of tests are used together in an effective monitoring program. Dipslides, while slow and inaccurate, can provide additional information as to the types of microbes in the water system when high ATP values are obtained.

ATP testing is typically used in several critical applications in industrial water systems. In routine testing of cooling towers and chilled loops, ATP Total testing can give a rapid indication of the level of microbial control in the system. After a recent addition of biocide or in conditions where water from a polluted source is continuously dosed with biocide, the Total and Free ATP tests provides a much better indication of the biocide efficacy. ATP tests can also be very useful in the detection and clean up of biofilms within water systems.

Total ATP: "Snap-shot" of Microbial Control.

Total ATP tests can be used to give an immediate "snap-shot" of the level of microbial control within a water system. While the Total ATP tests cannot discriminate between ATP from living microbes and ATP from cells that have recently died, high levels of total ATP indicate a system with poor microbial control. For recirculating systems general guidelines have been developed for the interpretation of Total ATP tests in this type of application.

	Open Recirculating (e.g. cooling towers)	Closed Recirculating. (e.g. chilled loops)
Pass	< 300 RLU	<200 RLU
Caution	300 – 750 RLU	200 – 500 RLU
Fail	> 750 RLU	> 500 RLU

Systems testing in the "pass" zone are under good microbial control. Towers that test in the Caution or Fail zone should be re-tested to ensure that the high results were not simply a "false-positive". If the high readings are repeatable the following steps can be taken:

1. Check the biocide tanks and pumps to ensure biocide is being added to the system.

2. Check the history of biocide addition – if biocide or biodispersent have recently been added, this could indicate the presence of a significant biofilm within the system. A Free ATP test can be used to determine if there are high levels of living microbes in the system (low free results) or if the high Total ATP readings are reflecting the dead cells from the biocide treatment (Free ATP ≥ Total ATP). The section below on Biocide Efficacy provides additional information on this approach.

3. Check circulation within the water systems – stagnant systems do not distribute biocide throughout the entire water system.

If the biocide is being added correctly and there remains a high level of microbial contamination, it maybe necessary to increase the dose of biocide or possibly switch to another biocide. Biocide resistance is to be expected after continuous use of a single type of biocide after a long period of time.

Biocide Efficacy

It is important when evaluating a biocide program, monitoring a continuous biocide addition to a contaminated source, or in switching biocides to obtain an indication of how effective the biocide is in eliminating microbial contamination. This can be accomplished quickly and easily using a combination of Total and Free ATP tests.

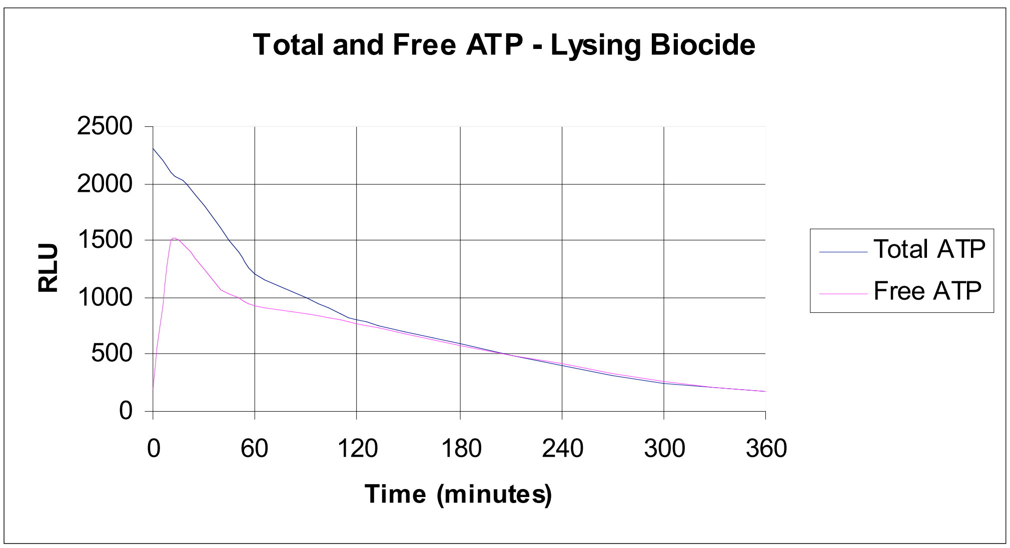
As mentioned earlier in this article, total ATP tests measure the "total" amount of ATP in the system, including the ATP that is free in solution as well as the ATP that is bound up in living cells. Free ATP, in contrast, only measures the ATP that is "free" in solution, i.e. all ATP except that in living cells. The difference between the two represents the ATP contribution from living organisms:

$$ATP_{total} = ATP_{Free} + ATP_{microbial}$$

$$ATP_{Total} - ATP_{Free} = ATP_{microbia}$$

In assessing the efficacy of biocides it is important to recognize that different biocide mechanisms will produce different ATP effects on the microbes treated. A few examples will demonstrate this phenomenon.

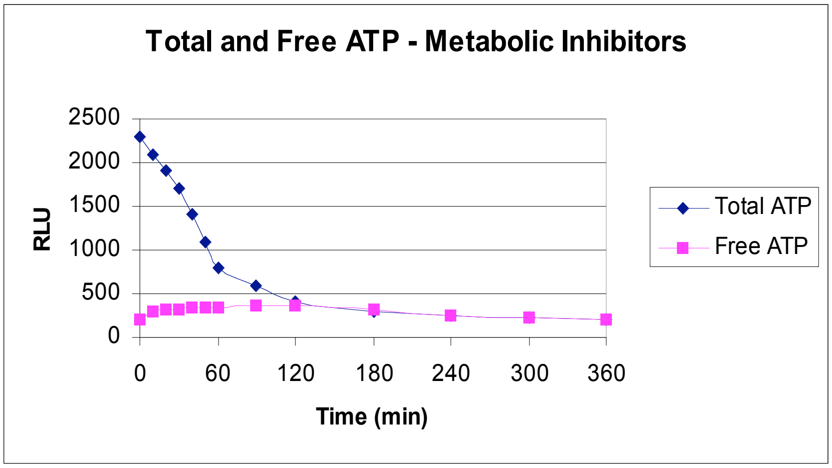
Example 1: Slug-fed Lysing Biocide



In Example 1, a biocide that acts by breaking open cells is added to a water system with high levels of microbial contamination. Lysing biocides such as quats, gluteraldehyde, and oxidizing biocides (chlorine, bromine, ozone) typically produce a rapid increase in free ATP and a gradual diminishing of Total ATP. In this example, the Free ATP rises rapidly within hours after addition of the biocide. The Free ATP is equivalent to the Total ATP indicating that essentially all of the ATP in the system is from non-microbial sources (i.e. most microbes are dead and have released their ATP to the environment).

In this type of biocide program, efficacy is determined by the convergence of the Free and Total ATP readings. Failure of the Free ATP to rise and meet the level of the Total ATP indicates that there is a population of microbes that have not been killed by the biocide addition. In most systems, the Total and Free ATP should converge within 24 hours of slug-feeding the biocide. This time period will be affected by the size of the water system, flow rate, level of initial contamination, and the type/combination of biocides used.

Example 2. Slug-fed Metabolic Inhibitors



In contrast to the lysing biocides, many non-oxidizing biocides act as metabolic inhibitors. These types of biocides cause a rapid decrease in the total ATP with little initial impact on the Free ATP levels. The reason for the different effects of Metabolic Inhibitors is that they act by poisoning the systems within the microbes that produce ATP without affecting the cellular structure of the bacteria. The results is that the Free ATP typically will not rise significantly while the Total ATP will drop significantly within a few hours. Note, however, that in this case low ATP may not accurately reflect cellular death, as energy drained bacteria may be revived if the metabolic inhibitors are removed. However, the rapid drop in ATP is a good indication of the activity of the metabolic inhibitors and cell death will follow rapidly.

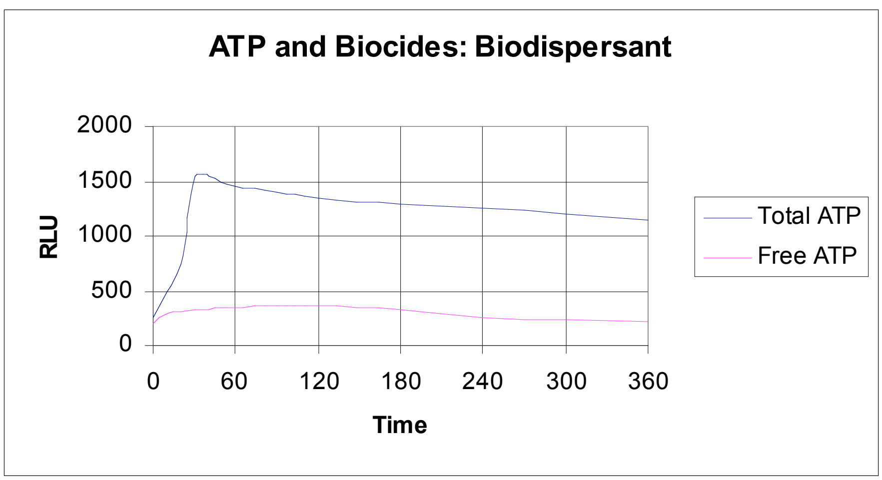
There have been some reports of Total ATP actually increasing in the first few hours after the addition of a metabolic inhibitor. While unexpected, this can occur with some metabolic inhibitors as the cells rapidly generate ATP in an effort to "restart" the cellular processes inhibited by the biocide. Regardless of the initial effect, the Total ATP should decrease over the first 24 hours of biocide addition.

With metabolic inhibitors, efficacy is indicated by a decrease in Total ATP to the "pass" zone within 24 hours. Note that this result is also observed with lysing biocides and may be used in lieu of the convergence end point. Additionally, there is typically a convergence of Total and Free ATP in the metabolic inhibitors as well, though this convergence may not occur for several hours after addition of biocide.

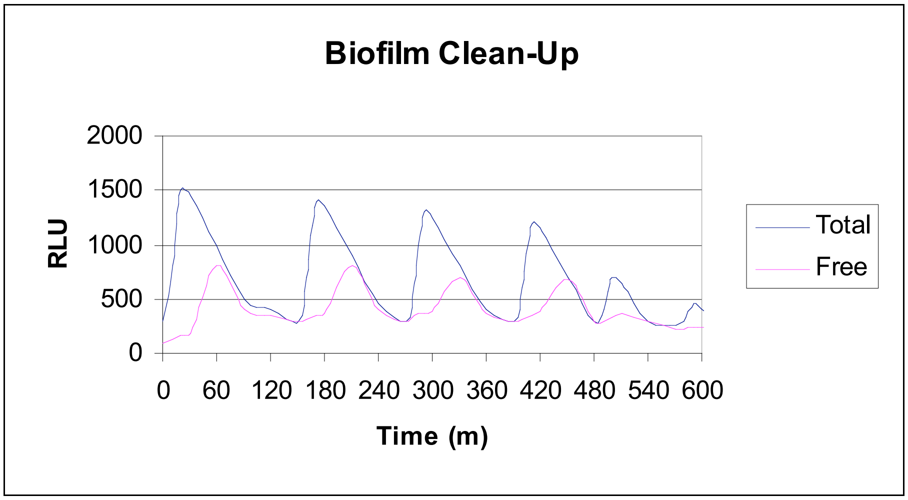
Biofilm Detection and Clean-up

Even with the best of biocide programs, it is almost impossible to completely prevent the eventual formation of biofilms in water systems. The addition of a biodispersant is a necessary part of an effective biocide program. However, the ability to track the effectiveness of this treatment has been extremely difficult. The use of dipslides, pour-plates, or other plating methods tend to underestimate the biomass released following biodispersant addition as the material released is often flocculent masses of several hundred, thousand, or hundred thousand cells adhered together. Regardless of the number of cells in a mass, a single colony per mass is the most that will grow on an agar surface.

Using ATP testing provides a rapid, simple method for the detection of biofilms and the evaluation of biodispersant efficacy. When biofilms are present within a system there is typically a rapid rise in Total ATP following biodispersant addition. The rise in Total ATP is due to the biodispersant transferring cells from the surfaces of the water system into the bulk water. Depending on the level of biocide within the bulk water there may or may not be a corresponding rise in Free ATP as the cells that were formerly protected within the biofilm are exposed to the biocides in the bulk water.



Once a biofilm is detected, it must be cleaned up to protect the system, enhance thermal efficiency, and prevent microbial induced corrosion. The most common and effective approach is to alternate the addition of biodispersant and biocide. Tracking total and free ATP during this process can indicate the efficiency of the clean-up effort and help determine the proper timing to maximize the biocidal activity.



As biofilm is released by a biodispersent, there is a corresponding rise in the Total ATP of the bulk water. When this rise has peaked, biocide can be added to effectively kill the circulating biofilm as well as the newly exposed layers of the biofilm. Effective kill of these cells can be detected as a convergence of Total and Free ATP readings. This cycle can be repeated several times until a post-biodispersent rise in Total ATP is not observed.

Conclusions

Rapid ATP testing is a vital part of an effective Microbial Monitoring System. The ability to immediately gauge the level of microbial contamination and the effect of biocides and biodispersents allow proactive steps and rapid response to the current microbial situation, rather than reacting to the conditions present a few days ago.

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