Water Talk

ATP Testing

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INTRODUCTION

Owners and operators of water cooled systems are constantly concerned with the cost and efficacy of microbial control. Treatment costs can explode if the biocides are overfed. Underfeed can lead to serious contamination issues.

Unlike other portions of the chemical treatment program where simple tests can determine whether there is sufficient treatment, biocide efficacy must be confirmed by observing the results of the treatment. Of course, in the case of biocides, it could take weeks or months before there are any visual indicators of a failing biocide program. By then much of the damage is done. The only solution at that point is a costly cleanup. Therefore, there must be an effective way to determine the efficacy of the biocide program, which is timelier than sitting around waiting for something bad to happen.

DIP SLIDE (& POUR PLATE) TESTING

Traditionally, water treaters have relied upon dip slides as the mode of choice for confirming the effectiveness of a biocide program. Some of the advantages of dipslides make them an attractive choice for many situations:

1. Dip slides are very cost effective.
2. Simple to deploy.
3. The results are easy to understand.
4. They require no special meters.
5. Results can be rapid enough for many situations.

While dip slide testing can be the avenue of choice for many applications, they do have some serious limitations:

1. Dip slides require time to incubate before results are ready. Typically 24 to 96 hours.
2. Each slide can only report the presence of a particular type of organism. Allows non-tested organisms to go undetected.
3. Standard agar slides do not report the presence of the more dangerous organisms (SRB, Nitrobacter, Legionella). These require more specialized slides, which the average water-treater does not routinely carry.
4. Residual biocides can continue to kill during the incubation period – False low results.
5. Dip slides and pour plate media permit the growth of only a small number of all organisms found in industrial water systems. The worst organisms do not typically grow on media in 36 hours (typical incubation time). This leads to a false sense of safety.
6. Dip slides results indicate only presence of motile (moving) organisms in a body of water. If there are slime masses, covering sessile organisms, then they will go undetected.

Clearly some of these limitations would preclude the use of dip slides, and therefore require a more time-efficient method to verify biocide effectiveness. This is where ATP testing comes into play.

WHAT IS ATP?

ATP (adenosine triphosphate) is present in all organisms. It acts as the universal unit of energy used in all living cells. ATP is produced and/or broken down in metabolic processes in all living systems. Processes such as photosynthesis in plants, muscle contraction in humans, respiration in fungi and fermentation in yeast are all driven by ATP. Therefore, any water filled system, that has a resident microbio population, will contain some level of ATP. An ATP luminometer (in conjunction with an ATP swab) uses bioluminescence to detect residual ATP as an indicator of cleanliness. The presence of ATP in a water sample indicates the presence of contamination, and/or bacteria. This implies a potential for the system to harbor and support microbial growth. Furthermore, the amount of ATP present in a body of water is directly proportional to the amount of biofouling present in the system. Simply stated, as the number of bugs increases, so does ATP.

ATP is determined using swabs which are treated with a special chemical derived from fireflies. When this chemical is exposed to ATP it will fluoresce (bioluminesce) in proportion to the amount of ATP present. This light is detected by the ATP meter (luminometer) which reports the result as Reference Light Units (RLUs).

ATP Luminometer and Swab

TYPES OF ATP

There are two basic tests for ATP. Free ATP and Total ATP. The difference between the two swabs is that the Total ATP swab has an additional chemical extractant, designed to lyse (break apart) the living microbio cells, causing them to release their interior ATP. Thus the Total ATP swab will report all of the ATP present, whether the ATP is freely floating about, or whether it is locked within a biomass.

Free ATP is the ATP that is present in a water sample, which is floating freely about in the sample. It is not bound up inside an organism. To test for Free ATP, you must use an ATP swab that is prepared differently from the Total ATP swab. The Free ATP swab does not contain the extractant chemical and will therefore only detect the presence of freely floating ATP.

Free ATP & Total ATP Swabs

TOTAL ATP, A SNAPSHOT

The most commonly performed ATP test is the Total ATP. Total ATP provides an immediate snapshot of the level of microbial control in a water system. While the Total ATP test cannot discriminate between ATP from living organisms, and ATP from cells that have recently died, it is still a very good indicator of system cleanliness. A high level of Total ATP is an indication of poor microbial control. General guidelines for water systems are as follows:

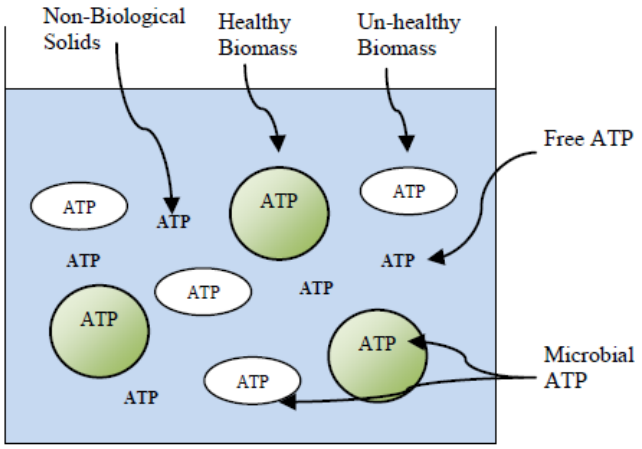
Total ATP, RLU		
	Cooling Towers	**Closed Loops**
Pass	< 30	< 20
Caution	30 – 75	20 – 50
Fail	> 75	> 50

MICROBIAL ATP AND BIOCIDAL EFFICACY

One way to determine if a particular biocide feed program is effective at killing microbes in a water system, is to determine the level of Microbial ATP before and after a feed event. Microbial ATP refers to the ATP that is contributed by biomass, (living cells). There is no means to perform a direct test for microbial ATP. It must be calculated from the Total ATP and the Free ATP levels.

Free ATP: ATP freely floating about
Microbial ATP: ATP contributed by living cells
Total ATP: Microbial ATP + Free ATP

Where: $ATP_{microbial} = ATP_{Total} - ATP_{Free}$



Microbial contents of a typical water sample

INTERPRETING ATP READINGS

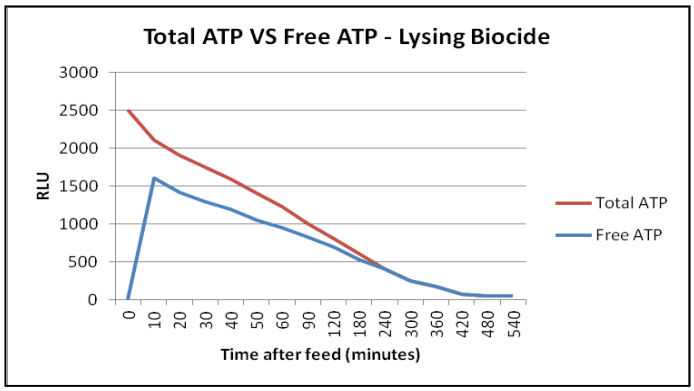
In general, a high *Total ATP* with a corresponding low *Free ATP* is typical of systems with poor microbial control. These two conditions indicate a high relative *Microbial ATP* value. Take a system with a Total ATP reading of 3,000 RLU (RLU = Reference Light Units), and a Free ATP reading of 50 RLU. This would mean that the Microbial ATP is 2,950 RLU (Total - Free). Thus the Total ATP consists of mostly ATP generated by living cells.

HOW DO BIOCIDES AFFECT ATP?

Lysing Biocides - Biocides that will break apart the cell are called Lysing biocides. Examples of lysing biocides include:

- Oxidizers
- Glutaraldehyde
- Quats

These types of biocides will cause an increase in the Free ATP, as the living organisms are destroyed, and their cell walls are broken apart. This action causes their ATP to become released to the water environment, which causes an increase in the Free ATP (could also cause an increase in the Total ATP).

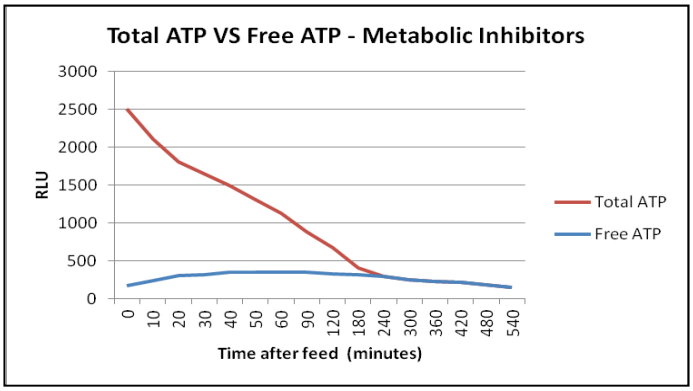


With lysing biocides, the efficacy of the program is determined by the convergence of the Free and Total ATP readings. This is the point at which all of the ATP present in the water system is NOT due to microbial sources (most of the microbes are dead and have released their ATP to the water).

If the Free ATP and the Total ATP levels do not eventually converge, then that would indicate that that there is a population of microbes that have not been killed by the biocide addition. The convergence can sometimes take as long as 24 hours after a biocide feed event, depending upon the kill time of the biocide.

Metabolic Inhibitors – Many types of non-oxidizing biocides are metabolic inhibitors. These will not break apart the cell wall; rather they poison the organism without affecting the cellular structure. The result is little or no change in the Free ATP reading. Examples of metabolic inhibitors are:

- Isothiazolin
- DBNPA
- Terbuthylazine

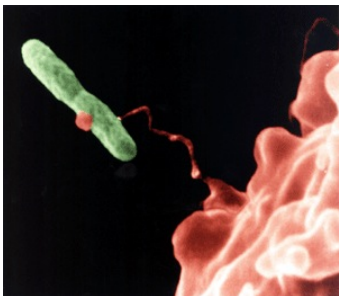


It is possible for Free ATP to increase after the addition of a metabolic inhibiting biocide. This can be explained as the cells are rapidly generating ATP to "restart" the cellular metabolic processes inhibited by the biocide.

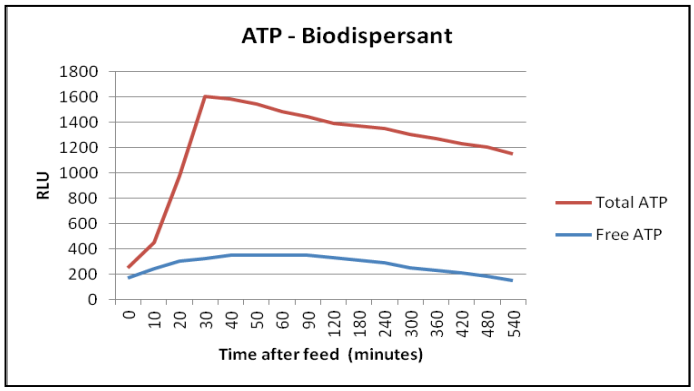
With metabolic inhibitors, the efficacy of the program is indicated by a gradual decrease in the amount of Total ATP, continuing until it drops to the "pass" zone. You might also see the same convergence of the Free and Total TP readings. As with Lysing biocides, this time to convergence would be a function of the kill time of the particular biocide used.

BIOFILM DETECTION

Our dispersant (4712) used to disperse biofilm is a necessary part of an effective biocide program. By using an ATP meter it is possible to track the effectiveness of a biocide program that includes a dispersant.



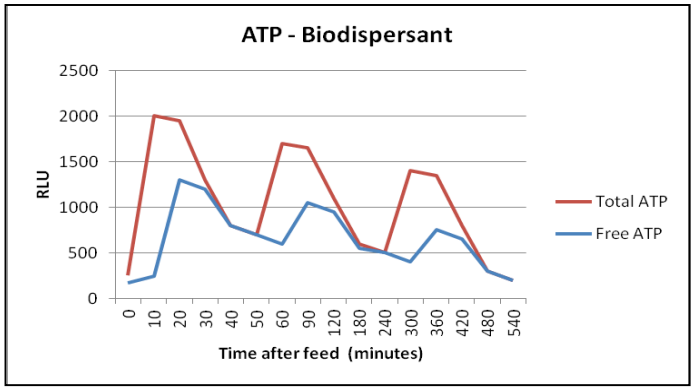
Dipslides and pour-plates will tend to underestimate the biomass released after a dispersant addition, as the material released is often masses (hundreds/thousands) of microbial cells adhered together as a single colony forming unit. Regardless of the number of cells in a mass, a single colony forming unit per mass is all that will form on the agar surface. In these cases the Total ATP and Free ATP readings can confirm the presence of a biofilm



Assuming you have fed a biocide and are getting very low ATP readings, in the pass zone. If you were to feed a dispersant, you could see the Total ATP readings rise, with little change in the Free ATP readings. The rise in the Total ATP reading is due to the dispersant transferring the microbial cells from within the slime and biofilm mass into the bulk water. If there is enough lysing biocide in the system, the Free ATP could rise, as the biocide acts to kill of those newly freed microbes.

BIOFILM CLEANUP

If the presence of a biofilm is confirmed, then it can be cleaned up, using the dispersant and a biocide in an alternating fashion, while tracking with an ATP meter. This will indicate the efficiency of the cleanup and optimize the timing of the biocide additions. The procedure is as follows: Add the dispersant. As the Total ATP readings increase and reach a peak, add the biocide. As the Total and Free ATP readings converge and drop, add more dispersant. Track the rise in Total ATP to its peak, then add more biocide. Continue this process until the Total ATP no longer increases after the dispersant addition.



CONCLUSION

While dipslides have many advantages, their major disadvantage is the long delay between sampling and results. ATP testing is very rapid, and therefore it can be an effective part of a microbial control program. The ability to rapidly determine the effects of biocide and dispersant additions allow for instantaneous corrective actions to be implemented.