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THE ATP ASSAY, A METHOD FOR MEASURING BIOLOGICAL ACTIVITY IN INDUSTRIAL WATER

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Biological control is a key to good corrosion and deposition control in units using industrial water. The challenge in preventing microbiological fouling is to detect a problem early enough to prevent unpleasant consequences. Microbiological populations are difficult to measure, quickly to change, and hard to control. The determination of total ATP by the luciferin/luciferase enzymatic reaction may be used as an indicator of water microbiological quality. This technique has the advantage of rapid measurement of all of the microbiological organisms in the sample, in comparison with the traditional colony count techniques, which determine only aerobic bacteria and require long incubation periods. Industrial water samples drawn from open recirculating cooling systems were analyzed for: colony forming units (CFU) representing the microbial content and Relative Light Units (RLU) representing the total ATP content of samples. Water samples with high RLU and low CFU contain greater amounts of non-bacterial species (molds, algae, etc).

INTRODUCTION

Biological control is a key to good corrosion and deposition control in units using industrial water. Microbiological fouling will eventually lead to a reduction in heat transfer efficiency and an increase in energy and water usage. The challenge in preventing microbiological fouling is to detect a problem early enough to prevent unpleasant consequences.

Biofouling is often difficult to detect because many bacteria are anchored to system surfaces, often shielded from biocide treatments by dead organisms, debris and cellular metabolic by-products (biofilm). Over a period of time, nutrition sources change. The prevailing wind in summer may blow grain dust into, *e.g*., a cooling tower. A hydrocarbon leak might occur. Either event can provide additional nutrients that increase overall microbiological populations and cause the rapid build up of damaging biofilms. Different nutrient types may also favour certain species. Similarly, a particular biocide treatment may be more effective at killing some species than others and this can create a stronger population of some selected species, since natural competition for food has been reduced. Most common microbiological testing methods only detect certain categories of organisms. Plate counts give a reading of the number of aerobic bacteria in a volume of water. Dip slides give a similar measurement of bacterial population, although results are semi-quantitative at best. Some of the most destructive species, such as sulphate reducing bacteria sloughed from the depths of a biofilm, are not detected by dip slides. Dip slides only reveal the presence of aerobic bacteria. Overall, microbiological populations are difficult to measure, quickly to change, and hard to control.

Most microbiological measurement techniques, such as dip slides, require 2 to 3 days for the culturing process to be completed.[1] By the time the system information is available, the results are history. For example, if a process leak 2 days ago created a significant microbiological population increase, the bacteria will now have begun to form a biofilm. The ATP assay is a method for measuring biological activity, based

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upon the fact that all living cells contain the molecule adenosine triphosphate (ATP); it is the principal medium of energy in all biological systems and is a key regulator of enzyme activity. The ATP assay is based on the bioluminescent reaction of the firefly: an enzyme, luciferase (Luc), in the presence of a cofactor, luciferin (LH_2), catalyses the hydrolysis of ATP, thereby producing yellow-green light.[2,3] The reaction is highly specific for ATP and can be summarized as follows:[4,5]

$$\text{Luc} + LH_2 + \text{ATP} \xrightarrow{Mg^{2+}} \text{Luc}\cdot LH_2^- \text{AMP} + PP_i \quad (1)$$

$$\text{Luc}\cdot LH_2^- \text{AMP} + O_2 \longrightarrow \text{Luc}\cdot \text{Oxyluciferin}^* + \text{AMP} + CO_2 \quad (2)$$

$$\text{Luc}\cdot \text{Oxyluciferin}^* \longrightarrow \text{Luc}\cdot \text{Oxyluciferin} + \text{light} \quad (3)$$

In the first step (Eq. 1), luciferase converts firefly D-luciferin into the corresponding enzyme-bound luciferyl adenylate. Firefly luciferase has extraordinary specificity for this nucleotide triphosphate. The adenylate is the true substrate of the subsequent oxidative chemistry. In fact, D-LH_2-AMP produced synthetically reacts with oxygen in the presence of luciferase to produce light emission identical to that obtained with the natural substrates D-luciferin and Mg-ATP. As Equations 2 and 3 indicate, the luciferase enzyme functions as a mono-oxygenase, although it does so in a very unusual manner without the apparent involvement of a metal or cofactor. In some way that has not been yet determined, luciferase amino acid residues are recruited to promote the addition of molecular oxygen to luciferin, which is then transformed to an electronic excited state oxyluciferin molecule and carbon dioxide, each containing one oxygen atom from molecular oxygen. Visible light emission results from the rapid loss of energy of the excited state oxyluciferin molecule via a fluorescence pathway. The very high quantum yield for this process (in alkaline solution, nearly each reacted LH_2 molecule emits a photon) reflects not only efficient catalytic machinery, but also a highly favorable environment for the radiative decay of an electronic excited state. The light emitted can be measured at its peak emission of 562 nm. One photon of light is produced for every molecule of ATP utilized, so that the total light output is directly proportional to the amount of ATP present in a sample.[6,7] As the emission of light is virtually instantaneous, the technique enables measurement of microbial activity within minutes – a distinct advantage over traditional colony count techniques, which require lengthy incubation periods.[8,9]

EXPERIMENTAL

The experiment was performed on treated industrial water samples drawn from open recirculating cooling system of an industrial gas producing company in the summer of 2005. The samples were analyzed for: colony forming units (CFU) representing the microbial content and Relative Light Units (RLU) representing the total ATP content of samples.

CFU was determined by counting the colonies grown on nutrient agar plates. The number of viable microbial cells was determined using the BIO-KOBE (Japan) colony counter. The result indicates the number of viable cells / mL of liquid sample.

Conventional ATP methodologies take about 30 minutes to carry out. We used a luminometer Betz Bioscan Monitor RHS 055, so the ATP analysis time was reduced to approximately 30 seconds. The luminometer detects light emitted and translates the light intensity into a measurement called a Relative Light Unit (RLU). The RLU number is proportional to the ATP in the sample and is, therefore, a direct measurement of the amount of living matter in the sample. The luminometer employs a sampling pen, which facilitates precise sampling, eliminating the need to mix and measure reagents, and functions as the measurement cell for the luminometer. A total ATP sampling pen consists of the following parts: -a stick for accurate sampling of the liquid to be tested, coated with an extractant, which release ATP from cellular material; the stick also transfers the sample into the cuvette; -a cuvette filled with test buffer for dilution, buffering and neutralization of the sample; -a reagent chamber sealed with aluminium foil, containing freeze-dried and stabilized luciferin/luciferase reagent.

RESULTS AND DISCUSSION

ATP bioluminescence is not a microbial count method but it is sensitive enough to detect the ATP content of individual cells in small numbers.[10] The concentration of ATP is directly related to the number of bacteria cells present in a sample. ATP is a chemical measurement only and does not measure the number of

bacteria. If pure cultures of bacteria are used and if they are assayed in the same metabolic state, a close correlation with the number of cells is possible (Figure 1).

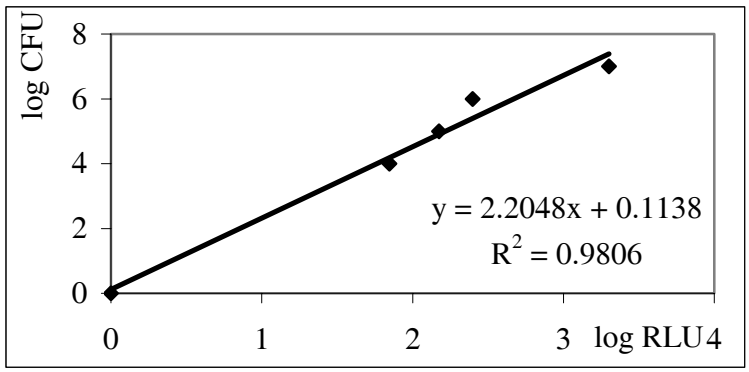

Fig. 1 – Correlation between the logarithmic RLU's and CFU's in laboratory conditions

Increases in microbiological population, as represented by the plate counts, are exponential. The luminometer, however, is not an exponential scale read-out (Figure 2).

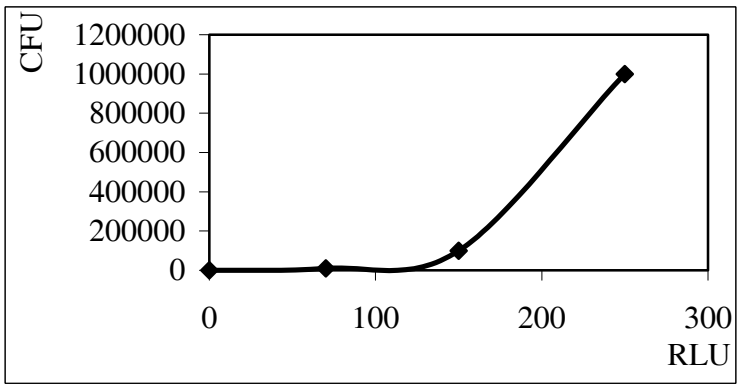

Fig. 2 – Bioscan RLU's as function of CFU's in laboratory conditions

Since ATP is so very sensitive, real world samples and real world microbial cultures yield total ATP values which do not correlate so well with the number of cells (Table 1).

Total ATP does not correlate to the CFU values in experimental conditions (Figure 4) as well as in the laboratory conditions, because the ATP method measures all of the microbiological organisms in the sample. Total aerobic plate counts measure aerobic bacteria which accounts for only a part of the ATP content. Fungi, anaerobic bacteria (responsible for corrosion), slow-growing bacteria, nitrifying bacteria, and various other bacteria are simply not detected by agar based methods.

Table 1

Results of the tests for monitoring microorganisms

Sample	RLU	Log RLU	CFU/mL	Log CFU
1	150	2.1761	6300	3.7993
2	36	1.5563	290	2.4624
3	24	1.3802	5	0.6990
4	22	1.3424	250	2.3979
5	14	1.1461	103	2.0128
6	37	1.5682	4	0.6021
7	82	1.9138	430	2.6335
8	100	2	2900	3.4624

The variation of the CFU is also exponential in the experimental samples (Figure 3).

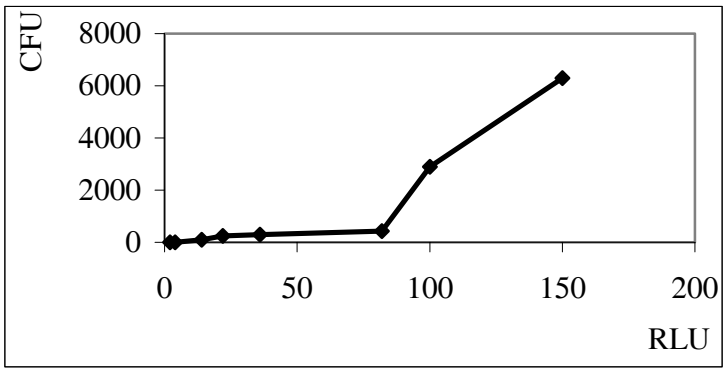

Fig. 3 – Bioscan RLU as function of CFU of water samples

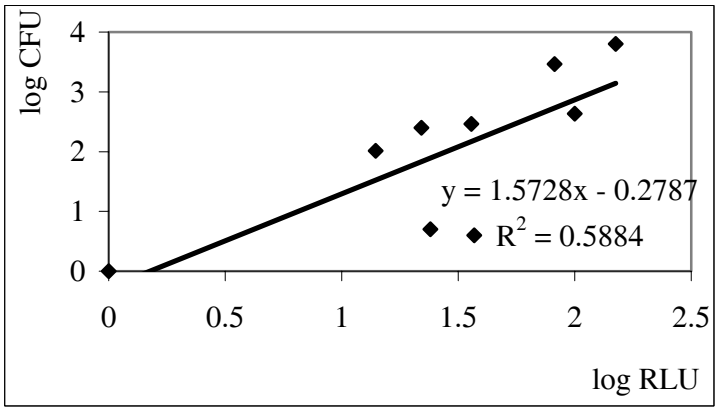

Fig. 4 – Correlation between the logarithmic RLU's and CFU's of water samples

Total ATP does not correlate to the number of live microbial cells (bacteria, fungi, algae, etc.). For example, algae contain much higher levels of ATP than bacteria. Even a small, near-invisible clump of algal cells, or organic film deposit contains high levels of ATP. The higher ATP of samples with low CFU indicates that the contribution of the non-bacterial species (molds, algae, etc) represents a greater percentage of the reading (RLU). We calculated the non-bacterial ATP as difference between total ATP (Bioscan reading) and bacterial ATP (Table 2). The total ATP (Figure 5) was calculated from the calibration equation of the luminometer: ATP = 0.0033·RLU-0.0123

Table 2
The values of the total, bacterial and non-bacterial ATP

Sample	Total ATP (ng/mL)	Bacterial log RLU	Bacterial RLU	Bacterial ATP (ng/mL)	Non-bacterial ATP (ng/mL)
1	0.4827	1.6752	46.7073	0.1418	0.3408
2	0.1065	1.0675	11.6640	0.0262	0.4827
3	0.0669	0.2660	1.8424	0.0062	0.1065
4	0.0603	1.0382	10.8615	0.0235	0.0669
5	0.0339	0.8632	7.2651	0.0117	0.0603
6	0.1098	0.2219	1.6651	0.0068	0.0339
7	0.2583	1.1453	13.8901	0.0335	0.1098
8	0.3177	1.5221	33.0095	0.0966	0.2583

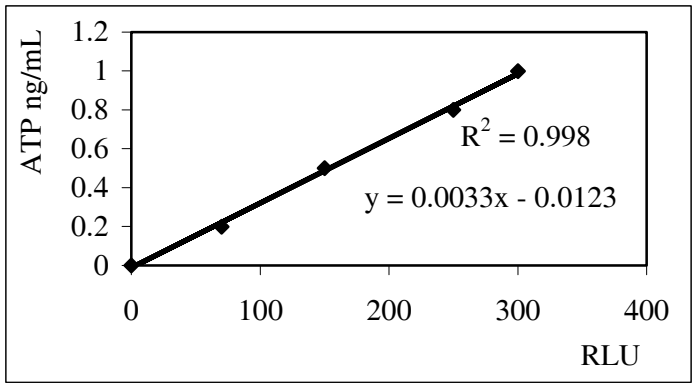

Fig. 5 – Relationship between ATP and RLU

The obtained data show that the most proportion of ATP content in the analyzed water samples represents non-bacterial microbes: moulds, algae etc. (Figure 6).

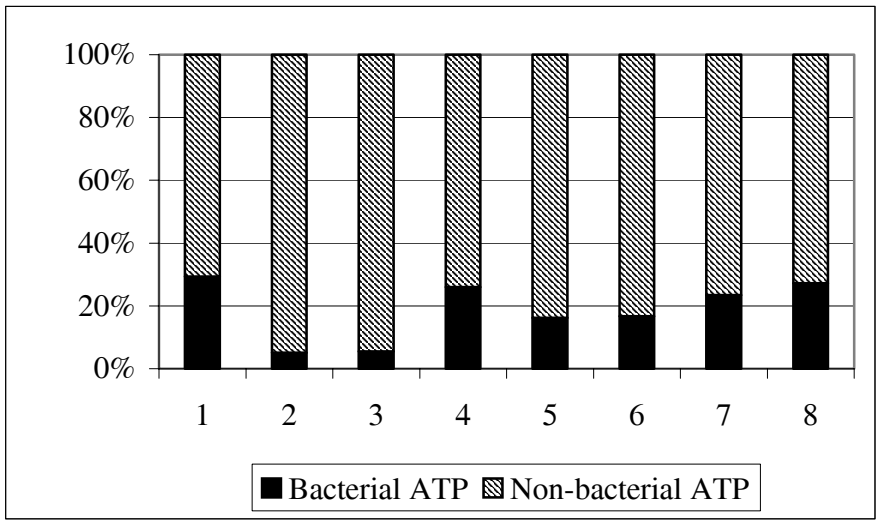

Fig. 6 – Relationship between bacterial and non-bacterial ATP

CONCLUSIONS

The determination of total ATP by the luciferin/luciferase enzymatic reaction may be used as an indicator of water microbiological quality. This technique has the advantage of rapid measurement of all of the microbiological organisms in the sample, in comparison with the traditional colony count techniques, which determine only aerobic bacteria and require lengthy incubation periods.

Industrial water samples drawn from an open recirculating cooling system were analyzed for: colony forming units (CFU) representing the microbial content and Relative Light Units (RLU) representing the total ATP content of samples. Total ATP does not correlate very well to the CFU values in experimental conditions, because the ATP method measures all of the microbiological organisms in the sample.

Water samples with high RLU and low CFU contain greater amounts of non-bacterial species (molds, algae, etc).

The advantages of the ATP method are: quick, highly sensitive, simple to use, cheap to use. The technique has a detection limit of 1pg ATP, which is equivalent to approximately 1000 bacterial cells. Due to these advantages, the ATP assay is a very convenient method for measuring biological activity in industrial water, especially for recirculating cooling systems, where a quick determination of the total microbiological populations is required.

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